

THE PATHOGENESIS OF POLIOMYELITIS

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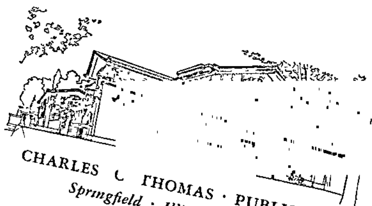
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THE PATHOGENESIS OF POLIOMYELITIS

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TO
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INTRODUCTION

FOR NEARLY a century, the pathogenesis of poliomyelitis has been a controversial subject and even today, with an imposing array of clinical, pathological and experimental observations at hand, differences of opinion persist among students of the disease, one group holding that the disease is primarily extraneural ("systemic," "general") and secondarily neural, and the other supporting the view that it is primarily neural with some secondary involvement of the extraneural tissues The comparative popularity of these two conflicting views has alternated in surprising fashion, and at the moment the first is again in favor It was first enunciated clearly in 1867 by C. F. Taylor[190] who remarked "My own belief is that the disease is essentially peripheric and that the great nervous centers are only indirectly and secondarily implicated " The second was adumbrated by Charcot[25] in 1870

The subsequent history, which need not be reviewed in detail here, was discussed with great care in 1941 by Goodpasture,[85] with particular attention to the pathological and experimental data that had been accumulated up to that time His summation was as follows "Experimental poliomyelitis in monkeys has thus equipped us with many new facts and novel points of view with which to reconsider the pathology and pathogenesis of the human disease Not only has it demonstrated the general character of the etiological agent, but it has confirmed the impression of many that the disease is primarily an infection of the nervous system, and not a secondary or accidental involvement of it following a general extraneural infection, that the nerve cells themselves are the primary seats of injury and not merely secondarily damaged by inflammatory exudation and infiltration, that the infectious agent is located within

the neurones themselves spreading mainly by means of the infected processes of nerve cells rather than through the body humors."

Observations made or amplified since 1911 have not shaken these conclusions in so far as they concern the neural character of the process in the central nervous system; that is, the strict neurocytotropism and axonal transmission of the virus therein However, certain pathological and experimental observations, mostly recent, have now led some students of the disease, notably Paul,[155] Jungeblut,[119] and Bodian,[11] to revive the old hypothesis, formulated with particular explicitness by Draper[32] in 1917, of primary extraneural infection with secondary extension through the blood stream into the central nervous system. Among these observations the most important are: the presence of lesions and sometimes also of demonstrable virus in certain "extraneural" tissues, notably cardiac muscle[119, 151] and lymph glands,[160, 197, 211] the occurrence of viremia, apparently with some regularity, in apes[11, 100] and human patients[17, 103] during the early stages of the infection, the demonstration of viral excretion and specific antibody formation, in apes and human subjects[129, 150] with asymptomatic infection after feeding, and the successful cultivation *in vitro* of poliomyelitis virus in various tissues free of nerve cells [34, 205]

Bodian's[11] current concept of the pathogenesis of poliomyelitis embodies three successive steps: 1) an "alimentary" phase in which the virus becomes implanted in the "alimentary mucosa" (not otherwise defined in terms of the exact tissues involved), 2) a "vascular" phase, during which the virus enters the blood from the mucosa, and 3) a "neural phase" in which invasion of the CNS* from the

* The following abbreviations will be used in this article

CNS central nervous system

LRE lymphoid reticuloendothelial apparatus

CSF cerebrospinal fluid

blood stream occurs at a single site, possibly the area postrema in the medulla, and then spreads within the CNS entirely by neural pathways. In essence, Bodian's hypothesis regards poliomyelitic infection as strictly neurotropic within the CNS but not outside it.

The validity of this hypothesis and of other similar ones is, I believe, open to serious question in a number of respects, since it is partly based on certain inferences and assumptions that at present are either dubious or unproved. Among these the following may be mentioned: the inference that the results of tissue cultures are relevant to the problem of tissue-host affinities *in vivo*, the assumption that viral excretion in the alimentary tract (pharynx, intestine) is proof of viral multiplication in the mucosa, the inference that viremia *per se* constitutes evidence of infection of extraneural tissues, and the view that infection of the CNS is solely due to direct invasion from the blood-stream.

Something should be said about the controversy over the portal of entry. For several years up to the latter 1930's it was widely believed that the virus first gained access to the CNS by way of the olfactory nerve system, primarily infecting the olfactory nerve cells in the upper nasal mucosa and thence spreading by axonal propagation through the CNS. This thesis was elaborated in my review[37] of 1933, in which an attempt was made to explain the whole disease process on the basis of strict neurotropism and axonal spread of the virus. A few years later as a result of observations by Horanyi Hechst,[85] Swan,[157] Sabin[149] and others showing that in the human disease the olfactory bulbs rarely if ever contain significant lesions, it became evident that this was not the normal pathway of entry. This did not however, justify exclusion of entry by way of other peripheral nerves of the respiratory and alimentary surfaces. Observations that such entry can occur will be presently discussed but, it is to be noted, do not exclude

other means by which the virus can reach the interior of the body.

The author and his associates have conducted experiments and published a series of papers, most of them since 1910, on which much of the following review is based. Some of our observations are published here for the first time. Our work has been oriented largely to the mechanisms involved in the initial infection and the pathways followed by the virus during invasion and excretion. Some questions, of course, remain to be answered. Nevertheless, it is felt that the present state of our knowledge is such as to justify another attempt at analysis and synthesis. Herein the role of the peripheral nervous system, hitherto largely neglected or minimized, has been carefully taken into account as one *of the major pathogenetic features of the disease.* In general, my aim has been to formulate a unitarian concept of poliomyelitic infection and, with as little theorizing as possible, to correlate the established facts.

Our experimental work from 1910 to 1953, as well as the preparation of the present review, has been aided by grants from the National Foundation for Infantile Paralysis, Inc.

H. K. F.

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A LARGE proportion of the experimental and histopathological investigations on which most of the present article is based was performed by Miss Rosalie J Silverberg, my first assistant from 1910 to 1952, and by Mr. Luther Dong, my second assistant from 1910 to 1953. The fact that their names appear on most of the papers imperfectly reflects the importance of their contributions, which were always marked by rigid standards of accuracy, dependability, and thoroughness, as well as by a high degree of competence For their faithful and loyal devotion I am deeply grateful My thanks are also extended to Mr Brayton Wilbur of San Francisco. In 1916 when the supply of cynomolgus monkeys, which were essential to the continuance of our experiments, had been shut off by World War II and its aftermath, Mr Wilbur arranged as a personal favor to me, to have his firm organize, on a cost basis, the collection of the same type of monkey from the Philippines Thus supplied until other arrangements could be made, we were able to resume our experimental work nearly a year sooner than would otherwise have been possible Finally, I offer my thanks to Dr Harry M Weaver, who was Director of Research of the National Foundation for Infantile Paralysis, Inc during almost the entire period covered by our studies, for helpful advice and assistance given me on many occasions

H K F



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PROPERTIES OF THE VIRUS

THE PATHOGENESIS of viral diseases in general and of poliomyelitis in particular must be considered, and can only be understood, in the light of certain general principles These have recently been simply and cogently described by Delbruck According to him, as reported by Adams,[50] the biological process involved in viral infections consists of three different phases or states of the virus, "the infective, vegetative, and provirus states In the infective state the virus is extracellular, metabolically inert, resting between cycles of reproduction It is extracellular virus which has been most extensively studied by physical, chemical and immunological techniques, and which is best known for its accessibility It is, however, of less interest to biologists than the intracellular states in which virus demonstrates some of the properties of a living organism In the vegetative state, the virus is intracellular, virus reproduction occurs, genetic changes of the virus take place, and the host cell metabolism is so disorganized that the pathology of the virus diseases is produced In the provirus state, the virus coexists with its host cell in a symbiotic relationship in which the virus assumes the *de facto* role of a genetic unit of its host cell The provirus protects its host cell against the attack of related viruses, profoundly affects the genetics of its host cell, and is the natural method of storage and preservation of viruses"

In poliomyelitis, the infective or extracellular state is represented by free virus in the pharyngeal secretions and stools in the blood, and probably in the fixed macrophages of the LRE after removal of virus from the blood or lymph The intracellular state is reflected in the chromatolytic and necrobiotic changes in the nerve cell which are a constant feature of the disease. While Howe and Bodian[109] have ex-

pressed the opinion that virus does not multiply in the axons, the fact that axoplasm is an integral part of the neuronal cytoplasm stands in favor of viral multiplication in the former, and, indeed, it is difficult to understand the centripetal and centrifugal migration of virus in the axons unless it actually does occur. There is no clear physiological evidence of two-way or alternating flow of the axonal contents but only of a very slow centrifugal movement, measured by Weiss and Hiscoe[204] at about 1 mm. a day, which is about one-fiftieth of the rate of centripetal migration of virus in peripheral nerve as measured by Bodian and Howe.[15]

Whether under ordinary conditions intracellular multiplication of poliomyelitis virus can occur in non-neural tissues *in vivo* is an interesting but actually unproved question. Many viruses are so highly specific that they can infect only one particular kind of cell in a single animal species and there are indications (brought out in other parts of the present communication) that poliomyelitis may fall in this category. Hyperplastic and necrotizing changes do, however, occur frequently, though not constantly, in the LRE,[160, 187] although virus itself is recoverable from it much less often.[211] The function of the LRE is largely defensive: the reticulum removes circulating pathogenic organisms from the blood and destroys them, while the plasma cells concurrently produce specific antibodies which are discharged into the blood stream as gamma globulin. It seems highly improbable, in view of their phagocytic and destructive properties that the virus could multiply even briefly within the cells of the LRE. The virucidal power of the reticulum must be considerable and its action rapid, since Lennette[illegible] has shown that when virus is injected intravenously in large amounts it can be recovered from the spleen for only a short time thereafter. The pathological

changes in the LRE may, in part, represent Selye's[144] alarm reaction, in which necrosis plays a conspicuous part

The provirus (latent," Horsfall's "steady") state, which may persist for a long time in infected but surviving and essentially intact host cells, is thought to be responsible for the long enduring immunity characteristic of many viral infections, including poliomyelitis Possibly the intranuclear inclusions that are occasionally found in neurocytes in poliomyelitis subjects may represent one aspect of this state

The cycle of cell invasion and of multiplication and release of virus in and from cells has been described by Horsfall[94] as occurring in five successive steps

1 Virus-cell union, assisted by a cofactor (tryptophane, etc);
2 Cell surface alteration (RBC agglutination, etc),
3 Viral penetration into cell,
4 Viral multiplication, in which the intracellular metabolism engages,
5 Release of viral particles from the cell

It should be noted that Step 1 of the invasive process is dependent upon an extracellular factor or factors

Step 5 is followed by repetition on a geometrically increasing scale of the same process (chain-reaction) larger and larger amounts of virus being released extracellularly into the environment to invade more and more cells This would correspond in poliomyelitis with the processes of excretion, reinvasion and viremia The invasion of the telodendra of axons from the mucosal surfaces is presumably governed by the same conditions and factors, as is also the release of virus from them on the surfaces of the alimentary tract

While the taxonomy of poliomyelitis virus has not yet been officially decided, Rhodes[155] in a recent discussion has

suggested "that the biological properties of the virus of human poliomyelitis first isolated by Landsteiner and Popper (1908, 1909) are sufficiently distinctive to justify placing this agent in a genus by itself." He proposes that this genus, Legio, be considered as having a single species, L. debilitans, with three subspecies, distinguished by their specific immunological properties, as follows: I. Brunhilde, II Lansing; III Leon. The murine neuronotropic viruses, such as those of the Theiler group, Col SK, MM and EMC (sometimes called poliomyelitis-like, parapoliomyelitis, and pseudopoliomyelitis) are regarded as belonging in an entirely separate category. In the present article the three "subspecies" will be designated as types 1, 2, and 3.

In 1948 the Committee on Nomenclature of the National Foundation for Infantile Paralysis[28] proposed certain diagnostic criteria for the identification of poliomyelitis viruses which, with certain refinements (mainly immunological) are generally accepted today These criteria are based "first upon clinical and histopathological manifestations of the disease produced in monkeys, second, upon host range; third, upon immunological relationships, and finally, upon physicochemical properties of the virus "

Clinical manifestations in monkeys when present (they may be absent or escape detection) are neurologic and similar to those in the human disease; fever, tremors, "spasticity, followed in a day or two by paralysis The sequence of severe generalized tremors and flaccid paralysis is pathognomonic

The nature and distribution, taken together, of the lesions, in the monkey are highly characteristic, and essentially duplicate those of human poliomyelitis *These are confined to the nervous system* and consist of chromatolysis, neuronal necrosis, neuronophagia and, later, "outfall" of nerve cells In addition focal and diffuse infiltrations with

perivascular "cuffing" accompany nerve cell damage The latter is most conspicuous in the motoneurons, both of the cord and brainstem where the maximal and most constant involvement occurs The vestibular centers, reticular formation and the roof nuclei of the cerebellum are frequently involved, but the cerebral cortex (excepting the pyramidal areas) and the cerebellar hemispheres are almost exempt. Further details are given under Pathology The distribution of virus in the nervous system during the acute phase closely follows the distribution of lesions

The three types of poliomyelitis virus appear to produce the same clinicopathological picture Type 2 has rarely, if ever, been found responsible for epidemics Such differences as have been noted between strains (not necessarily of different types) appear to relate mainly to virulence (i e, invasiveness and severity), some being "mild" and some "severe" depending upon the severity of the disease they produce in experimental animals and to the ease with which the latter can be infected, particularly by the oral route[149]

The change that occurs during sequential passages of a given strain and its adaptation to the host appears to consist largely of heightened, less often of lowered and occasionally of fluctuating virulence, and not to the essential character of the disease produced It is questionable whether the experimental observations of Trask and Paul[197] of loss of capacity to infect by intracutaneous injection mean more than a simple reduction of invasiveness, since similar failures of sequential passage of recently isolated strains are by no means rare even by intracerebral inoculation It is interesting to note that minute amounts of the archetype of monkeyized virus, the Rockefeller MV (type 2), which had been through innumerable animal passages for about thirty years, was still capable of producing human infection,

with typical clinical manifestations.[136]

Primates are with rare exceptions the only known hosts suitable *in vivo* for direct isolation, but some strains (mainly of type 2) can be adapted by passage to certain rodents (cotton rats, white mice, occasionally hamsters).

The physiocochemical properties need not be reviewed here at length. The virus has an average size of about 30 mμ, is approximately spherical,[4, 152, 162, 163, 164, 172] is resistant to ether, susceptible to drying,[45] and does not agglutinate red cells.[131]

In considering the distribution of virus outside the nervous system in patients dying of poliomyelitis, it should

TABLE I

RESUME OF TESTS RECORDED IN THE LITERATURE FOR POLIOMYELITIS VIRUS IN LYMPH NODES OTHER THAN TONSILS IN FATAL HUMAN CASES

Condensed from Wenner and Rabe[198]

Lymph Nodes			
Cervical	*Axillary*	*Inguinal*	*Mesenteric*
1/10	8/19*	5/19*	6/76

Numerator positive test *Denominator* number of cases in which tests were made

* In 7 cases (1 positive) the axillary and inguinal nodes were pooled

be kept in mind that practically all "extraneural" tissues contain nerve fibers and in the course of infection, as will be shown later, the virus undergoes an extensive propagation throughout the peripheral nerves. As regards the "extraneural" tissues virus has been most frequently recovered from the tonsils,[111] occasionally even from patients without clinical poliomyelitis.[124] It has also been recovered fairly often from other LRE tissues, including the spleen. Wenner and Rabe's[11] recent study and compilation, reproduced in Table I shows the comparative frequency with which the various lymph node groups have been

found to harbor virus; the usually negative tests of the mesenteric nodes are notable. Sabin and Ward[174] have also made an elaborate search for virus in seven human autopsies, the results of which are shown in Table II from their report

The fact that lesions are sometimes, though exceptionally, observed in the skeletal and cardiac muscle in cases of poliomyelitis has led to a search for virus in them. Investigations on the point have been made by Jungeblut and Stevens[120] who recovered virus from human skeletal

TABLE II

DISTRIBUTION OF VIRUS IN HUMAN POLIOMYELITIS

Tissues Tested	*Results**
Adrenal glands	0/7
Salivary glands	0/7
Lymph nodes	
Cervical	0/7
Mesenteric	0/7
Axillary and inguinal	1/7
Nasal mucosa	0/7
Pharyngeal mucosa, tonsils	4/7
Ileum washed wall	3/7
Ileum, contents	2/7
Descending colon, washed wall	1/7
Descending colon, contents	6/6

* *Numerator* number of positive cases, *denominator* number of cases tested From Sabin and Ward.[145]

muscle once in thirteen attempts, using biopsy material obtained during the acute stage (tests on the same individual a little later were negative), and from cardiac muscle at autopsy in three of five attempts. The relevance of these findings to the question of extraneural tissue infection is obscured by the probability of peripheral nerve invasion, the presence of neuronal ganglia in the heart, and the possibility of blood contamination.

Poliomyelitis virus, following the studies by Enders and

his associates,[34] has now been grown in a considerable variety of tissue, mainly human and simian, in which no nerve cells are present, such as connective tissue, epithelium, testis, kidney and uterus In tissue cultures cytopathological changes are constant and characteristic, but are found mainly in fibroblastic and epithelial cells There is a striking lack of correlation between the tissue types that support growth in the flask and those that show lesions in the intact animal, including man. Thus, neither the collagenous nor the epithelial tissues nor any of the major extraneural viscera in living subjects infected with poliomyelitis show significant pathological changes with any regularity, whereas the only tissue that does show changes with some consistency in living subjects, the LRE, has not as yet been found capable of supporting viral growth *in vitro*. An interesting observation is that injection of poliomyelitis virus into the testis of monkeys fails to induce infection,[36, 121] whereas testis constitutes an excellent substrate for viral growth *in vitro*.[156]

The general absence of clinical and pathological correlations between the tissues providing such *in vitro* substrates and those affording clinical signs and pathological changes *in vivo* is striking, as illustrated by the fact that such manifestations as nephritis, orchitis, enteritis, collagen disorders, etc are not part of the poliomyelitis syndrome

In a discussion[42] of this lack of correlation published in 1951, I pointed out the sharp differences in the conditions of tissue culture and those obtaining in the intact animal with reference to the extracellular fluid e g., the removal of inhibitory substances resulting from washing of tissue fragments, the use of electrolyte solutions differing in composition from the normal mammalian interstitial fluid; the addition of material (ox blood ultracentrifugate) with

special cell stimulating properties to the suspending fluid. The following passage from Enders[33] summarizes the subject clearly. "The results of many studies with different viruses . . . have made it clear that the degree of pathogenicity exhibited by an agent for the intact animal is frequently not correlated with its capacity to increase in cultures from the tissues of such an animal, ... Extracellular inhibitory mechanisms present in the living animal may be eliminated in cultures, thus permitting multiplication."

The conclusion seems justified that *the results of tissue culture in vitro have no relevance at all to the problem of specific tissue affinities for poliomyelitis virus (or other viruses) in vivo*

While tissue culture is an unnatural process in reference to the intact animal, it is of great biological interest since it demonstrates that radical changes in cell environment can lessen or entirely remove the specificity of the cell-host virus relationship, permitting both viral invasion and multiplication to occur in normally insusceptible kinds of cells The experiments of Schwartzman and Aronson[143] show that hormones exert a similar effect in the intact animal: in these the administration of cortisone in large amounts to Syrian hamsters, a usually refractory species, enormously enhanced infectivity and also promoted viral multiplication in tissues such as the periadrenal fat never before implicated in poliomyelitis infection Lesions in muscle were also produced The search for extraneural lesions in experimental poliomyelitis had hitherto been consistently unfruitful, and the results obtained with cortisone strongly suggest that the not uncommon, though inconstant, presence of such lesions in fatal human poliomyelitis, notably in muscle and lymph nodes, may be due to severe stress accompanied by an

outpouring of adrenocorticotropin and adrenocortical steroids. If so, human extraneural infection with poliomyelitis virus may occur under special conditions that may be regarded in a sense as "natural" but, it is to be emphasized, *this would necessarily be a late, secondary phenomenon and certainly not part of the primary or even an early phase of the disease, such as the "minor illness"* The subject will be referred to again.

An important characteristic of poliomyelitis virus, and of many other viruses, is the self-limited and rather precarious foothold it obtains in the host tissues. This is shown in several ways the mildness and briefness of the majority of infections, the restriction of its affinities to a few types of nerve cells; the frequent recovery from infection of even the most highly susceptible cells, the *motoneurons, and the frequency of clinical recovery, partial* or nearly complete, of patients from paralysis Evidently, the adaptability of the virus to the host is commonly a tenuous one, and the host-cells appear to be capable of rapidly adjusting their structure or enzyme systems in directions adverse to the requirements of the parasite, thus establishing an enduring immunity.

Summary

In the intact animal, poliomyelitis virus has a highly selective affinity for neurocytes, among which only certain varieties are susceptible and among these there are striking gradations in susceptibility Axonal propagation of the virus is a basic and characteristic feature

A wider range of affinity can be artificially induced *in vitro* by drastic modifications of the normal extracellular environment *of the host cells, and in vivo* by administration *of cortisone in large amounts* Both these conditions permit infection of extraneural tissues to occur. The con-

ditions of tissue culture have no relevance to the natural disease. The results with cortisone might, however, have such relevance to late stages of severe infections, accompanied as they are by extreme stresses, but not to the primary and early, mildly symptomatic, phases.

As a rule, poliomyelitis virus obtains only a tenuous foothold in the host, usually the disease is self limited, mild and brief, but nevertheless produces a lasting immunity

EPIDEMIOLOGY: MODES OF INITIAL EXPOSURE

THE EPIDEMIOLOGY of human poliomyelitis and the methods by which the disease can be conveyed to experimental animals, particularly primates, have been extensively investigated. In experimental work "unnatural" (traumatic) as well as "natural" (atraumatic) methods of exposure find their counterparts in the human disease: the former correspond with certain comparatively uncommon post-traumatic human cases occurring after tonsillectomy, dental extractions, injections, etc., while the latter throw light on the etiology of the much larger moiety of ordinary cases without preceding gross trauma, both paralytic and non-paralytic. In the experimental animal, poliomyelitis can be readily induced by such "unnatural" methods as intracerebral, intraneural, intra- and subcutaneous, intramuscular and intraperitoneal inoculation, and direct exposures of the olfactory mucosa; and by such "natural" methods as inhalation and ingestion, including non-traumatic applications of virus to the oropharyngeal surface.

Surveys based on neutralization tests,[151, 193] recoveries of virus,[illegible] and age distribution of reported cases[103] indicate that a very large proportion of the general population must harbor poliomyelitic infection one or more times during the first two decades of life, and that the ratio of unrecognized (subclinical, mild) to recognized cases is very high, by some estimates 1000:1 or more. The total incidence is probably no less than that of measles.[103] *These data strongly suggest that the usually prevailing strains of virus under ordinary conditions are characterized on the one hand by high endemicity, infectivity and immunizing power but on the other hand by low invasiveness and*

virulence in the sense that they have a strong tendency to die out quickly in the host without extensive spread or severe injury. Immunity to type 2, for example, is very widespread but epidemics referable to it are extremely rare

This concept was found by us[82] to have an analogue in a laboratory in which rhesus and cynomolgus monkeys

TABLE III

LESIONS IN PERIPHERAL GANGLIA OF UNINOCULATED MONKEYS, RELATED TO LENGTH OF STAY IN LABORATORY

Ganglion	*New* No Animals	*New* %	*Old* No Animals	*Old* %
Gasserian	9		17	
Lesions present*		0		24
Neuronophagia		0		41
Nodose	9		17	
Lesions present*		11		24
Neuronophagia		0		41
Superior cervical sympathetic	9		17	
Lesions present*		33		71
Neuronophagia		0		35
Celiac	7		8	
Lesions present*		14		38
Neuronophagia		0		0

* Infiltrative lesions of more than minimal size, without neuronophagia
New in laboratory 3 days or less *Old* in laboratory 17 days or more

were housed and the only virus in use was that of poliomyelitis By comparing the extent and nature of lesions in peripheral ganglia in animals sacrificed shortly after reception with those in animals sacrificed after two or more weeks sojourn in the laboratory, an interesting difference was noted, as shown in the following table It appeared from these observations that a stay of seventeen

days or more in the laboratory with only casual and accidental exposures resulted in the acquisition of lesions, notably neuronophagia, indistinguishable from those of poliomyelitis, despite the complete absence of clinical manifestations of infection.

The chief sources of infection are the pharyngeal secretions and the stools of patients during the late presymptomatic, symptomatic and early convalescent stages, and the generally accepted view is that person-to-person contact with, or at least close proximity to, a carrier is the way in which the infection is usually acquired. Three principal modes of primary exposure therefore have to be considered: 1) *inhalation* of air contaminated by droplets expelled from the nose or throat, or of dust contaminated by sputum or feces; 2) *oral introduction* by means of fingers, food, drink or eating and drinking utensils contaminated with virus, mainly from fecal sources, and 3) *traumatic introduction* of virus through the mucous membranes or skin

In attempting to decide which of these are "natural" modes of primary exposure in man, the human and experimental data must be carefully correlated. Neglect of this precaution has led to serious errors of interpretation in the past Thus, the fact that apes can readily be infected by nasal instillation of virus or by inhalation does not *per se* prove that human poliomyelitis is acquired from the air, nor does the fact of pharyngeal and intestinal excretion by itself prove that it is acquired by ingestion Before experimental evidence becomes valid for human disease it must be found to agree with, and not contradict human pathological and other evidence In the following paragraphs relevant data on the various modes of exposure will be presented and an attempt at correlation made

Infection by Inhalation

Until recently, the view that poliomyelitis is an air-borne disease had been widely favored and even now has by no means been universally discarded. The positive evidence in favor of the respiratory route is almost entirely experimental. First demonstrated by Leiner and von Wiesner[117] in 1909 and abundantly verified since then, infection is readily induced in both monkeys and chimpanzees[106] by the introduction of virus-containing materials into the upper nasal passages. In all probability the same results would occur in man under comparable conditions.

Until 1941 practically all experiments on the respiratory route of infection by others as well as ourselves, had been directed to the olfactory route and done under decidedly artificial conditions—tamponade, preparatory lavage with acid solutions, introduction by dropper or catheter of fluids or stool suspensions containing virus—for which counterparts in ordinary human experiences are difficult to imagine. Further study of infection by the respiratory route obviously called for more natural conditions of exposure.

Wells and his associates[208-210] have shown that certain respiratory diseases, such as influenza, pneumonia, tuberculosis and probably measles, can be spread through the air by fine droplets expelled from the nose or mouth, containing the infectious agents. Such droplets dry rapidly and as 'droplet nuclei' float for considerable periods of time and for considerable distances in the air and probably constitute the major mode of contagion in diseases of this type. Larger droplets (Flugge type) fall quickly and are presumably of less importance. In 1941[45] and subsequently,[50] we explored droplet nucleus infection with poliomyelitis virus, using a completely atraumatic method

designed to simulate natural conditions of air-borne spread.

A fully enclosed monel metal chamber was constructed with four portholes through which the heads of monkeys could be placed, and into this chamber a virus suspension was blown through an atomizer reducing the suspension to fine droplet form which dried rapidly in the air chamber. The Per (type I) and OH (type unknown) virus strains were used. In some of the monkeys the olfactory area was blockaded with 1% zinc sulfate, as described by Schultz[152] and in others the olfactory mucosa was left intact. Histological examinations of the olfactory bulbs and other portions of the central nervous system were made in all animals to determine whether infection had entered through the olfactory nerve paths or elsewhere. The results of the experiments are summarized in Table IV.

TABLE IV
POLIOMYELITIS AFTER INHALATION

	Olfactory Blockade		*No Blockade*	
	Cynomolgus	*Rhesus*	*Cynomolgus*	*Rhesus*
Number exposed	10	35	7	7
Number developing poliomyelitis	4	4	6	5
Olfactory takes	0	2	6	5
Non-olfactory takes	4	2	0	0
% positive	40%	11%	86%	71%

The experiments demonstrated that both cynomolgus and rhesus monkeys (the former with much greater ease) can be infected by inhalation of virus both by non-olfactory and by olfactory routes. When the olfactory nerves in the superior nasal meatus are intact, infection occurs with regularity through these nerves, the olfactory bulbs showing typical lesions

In 1911 Neustaedter and Thro[18] reported the recovery of poliomyelitis virus from the floor of a sickroom, but no further studies appeared in the literature for the next forty years. In 1951 we[22] examined samples of dust from 118 houses in which poliomyelitis had recently occurred, using the method of high-speed centrifugation and intracerebral inoculation into cynomolgus monkeys All tests were negative The conclusion was reached that any virus eliminated by patients and incorporated in house dust fails to survive for two weeks or more, and possibly less.

To supplement and extend these observations, the effect of drying on poliomyelitis virus was investigated,[22] also in 1951, using the Lansing (Armstrong, type 2), and Wis'45 (type 1), strains.

The experiments were divided into three groups. 1) aqueous suspensions of virus 2) suspensions of virus in oropharyngeal washings from normal cynomolgus monkeys, and 3) cynomolgus stool mixed with Wis'45, and a known positive human stool In group 1, drying for periods of thirty minutes and more resulted in complete inactivation, when desiccation was complete. In group 2, a small amount of residual activity was found at four hours none at sixteen hours In group 3, inactivation was incomplete at sixteen hours and complete at twenty and twenty-two hours A review of the conditions under which the materials were dried showed that when dried *in vacuo* over $CaCl_2$ (that is, when drying was probably complete) rather than at atmospheric pressure, inactivation was prompt and complete On the other hand, when small amounts of residual moisture remained, inactivation was delayed and incomplete When virus-containing stools were dried and triturated to a fine powder, the tests were uniformly negative

These experiments permit us to conclude that complete

drying of poliomyelitis virus, such as might be expected to occur rapidly in the case of fine droplets expelled into the air from nose or mouth and somewhat less rapidly in the case of large droplets of pharyngeal mucus and of fecal material in the course of dust formation, causes inactivation within a relatively brief period. Residual moisture on the other hand prolongs its survival. These results provide some evidence against air-borne infection in poliomyelitis by droplet nuclei or dry dust.

Epidemiological evidence[170] also suggests that poliomyelitis is not an air-borne disease. It has long been recognized that paralytic cross infections rarely occur in hospitals where poliomyelitis patients are housed, either to nurses and doctors in attendance or to other patients, and it has been regarded as safe for poliomyelitis patients to be admitted to general hospitals and wards if ordinary precautions in disposal of stools are taken. Likewise, no increase in the incidence of poliomyelitis has been noted when schools are opened in communities where the disease is prevalent. Moreover, the disease itself is not commonly or characteristically accompanied by sneezing, coughing or nasal discharge which would tend to expel droplets into the surrounding air. Sabin and Ward[illegible] showed that virus could not be recovered from the saliva nor from anterior nasal secretions of patients, and it has been shown by others that nearly all the material expelled from the mouth and nose during sneezing consists of these materials.[illegible] The experiments of Ward and Walters[illegible] showed that when patients in the acute stage of the disease were masked and asked to cough, virus could only exceptionally be found on the masks even after violent coughing.

Since 1935, when Horanyi-Hechst[95] published his observations which were subsequently confirmed by Swan,[illegible]

Sabin,[188] and others, it has been well established that the olfactory bulbs of human patients dying of poliomyelitis rarely contain lesions* whereas those of experimental animals exposed by direct application of virus to the intact olfactory mucosa and as shown by our experiments[68] as above mentioned by inhalation of air containing virus-bearing particles almost uniformly do It seems almost certain—though not susceptible of direct proof—that lesions would occur in human olfactory bulbs if the olfactory mucosa were similarly exposed The correct application of the experimental results to human poliomyelitis would appear to be that under "natural" conditions the human olfactory mucosa is infrequently exposed to the virus because the latter is rarely air-borne in infective amounts and because virus-containing material in fluid or semi-solid condition rarely reaches that area

It seems fair to conclude that, with possible rare exceptions, poliomyelitis is not spread by way of the air, either in dust or in droplets

Infection by Oral Entry

The evidence for oral entry consists of 1) occasional epidemics traceable to food, 2) observations and inferences pointing to fecal contamination as opposed to air-borne spread, 3) experimental reproduction of infection

* In a few instances lesions usually infiltrative rather than destructive have been found in human bulbs and in one case Kessel and his associates recovered virus from them While these findings may have been due to a secondary invasion or centrifugal spread they leave the possibility open that exceptionally the olfactory pathway may serve as a point of entry of human infection Conditions under which this might occur are submersion of the head in contaminated water during swimming and regurgitation of contaminated pharyngeal mucus in the course of vomiting Actually, there is very little convincing evidence that the disease has been acquired in swimming pools

by the oral route, and 4) demonstration of lesions in human cases referable to entry from the alimentary tract.

Epidemiological evidence for infection from ingestion of food contaminated by carriers handling it or by flies is strongly suggestive, if not absolutely conclusive, in the case of a few explosive and localized outbreaks traced to milk. Eight such outbreaks were reviewed in the Milbank Committee's report (1932), of which four met certain criteria laid down by Stallybrass.[174] Lipari[141] has recently reported a local outbreak in which milk was under suspicion. In the outbreak reported by Goldstein, Hammon and Viets[72] in 1946, contamination of milk and possibly other foods by flies was incriminated. Raw vegetables and fruit have been held suspect by a few observers, but without satisfactory evidence. Gebhardt and Woodie,[80] however, found poliomyelitis virus in the stools of a peddler of such foods.

In regions where human feces is used for fertilization of soil, contamination of vegetable foods is inevitable and their consumption in an uncooked condition must afford a ready and common means of oropharyngeal and intestinal exposure. The discovery that flies can capture[55] and convey poliomyelitis virus from fecal sources[158, 174] has disclosed another potential method of food contamination for which direct confirmation has been supplied by the experiments of Ward, Melnick and Horstmann.[197]

There have been numerous experimental studies showing that oral administration of poliomyelitis virus will produce infection in primates, including man, although with considerable variations in frequency depending partly on the species of test animal and partly upon invasive properties of the particular strain of virus employed in the tests. The fact that rhesus monkeys (M. mulatta), for many years the species mainly used in experimental work

with poliomyelitis, are difficult to infect by feeding was probably responsible for the general disregard until recent times of the oral route as an important "natural" pathway of infection However, as early as 1912 Levaditi and Danulesco[139] had succeeded in infecting a monkey of the irus group (M. sinicus) by placing it in a cage in which the food pan and floor had been contaminated with virus Subsequent studies by Kling, Levaditi and Lépine,[127] by Saddington,[175] by Levaditi, Kling and Hornus,[140] by Sabin and Ward,[177] by ourselves,[61] and many others have clearly demonstrated that cynomolgus (M irus) monkey is readily infected by oral administration of virus Melnick and Horstmann,[150] and Howe, Bodian and Morgan,[110] have shown that the chimpanzee can be likewise infected, perhaps with even greater ease In both the cynomolgus monkey,[104] and the chimpanzee,[110] clinically inapparent infection ("carrier state"), with the development of specific immune antibodies has repeatedly been produced by simple feeding

Crucial evidence that human beings can be infected by the oral route has been supplied by Koprowski, Jervis and Norton,[129] in 1952, and by Koprowski, Jervis, Norton and Nelson,[130] in 1953, who fed a mild strain of the Lansing (type 2) virus to two groups of volunteers during a study of active immunization None of the subjects showed clinical signs of infection but most of them excreted virus in the stools and nearly all developed type 2 neutralizing antibodies

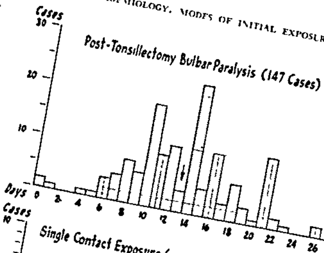
It is much easier to infect monkeys orally with some strains of virus than with others Thus, with Philippine cynomolgus we[61] were able to infect three of six with the Cam strain but in two of these second and third feedings were required, and Howe and Bodian[103] obtained only three takes in eighty-eight tests with this strain of virus

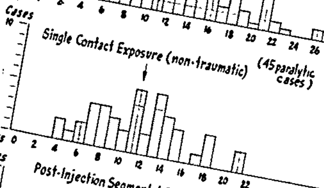
Using the Wis'45 strain (type 1) obtained from Dr. Paul F. Clark, of Wisconsin, who obtained results similar to ours, we were able to infect cynomolgus regularly (7/7) with a single feeding. With the Lansing strain it appears to be somewhat more difficult to obtain takes and a carrier state with monkeys and chimpanzees by oral administration The occasional success obtained with the orally less infective strains suggests that the differences are quantitative rather than qualitative.

Now that virus has been demonstrated in the blood of human patients the possibility of transmission of the disease from person to person by biting insects must again be taken into consideration In contrast to the arthropod-borne neural diseases (equine encephalomyelitis, etc.), evidence of such transmission of poliomyelitis is lacking and several experimental attempts[74, 126] to convey the disease by mosquitoes have failed. Most of the flies found to contain virus have been of the filth-feeding rather than the biting varieties [78]

Traumatic Introduction

That human poliomyelitis sometimes follows penetrating trauma, notably adenotonsillectomy[19] and injections, especially of antigens,[41] is well established. The causal relationship between such local injury and the ensuing paralysis can be demonstrated in three ways: 1) the interval between the two events corresponds with the usual incubation period of the disease with only rare *exceptions* (Figure 1), 2) the site of paralysis is correlated with the site of injury, and 3) chi-square tests[33] show the localizations of paralysis in the post-traumatic cases to be significantly different statistically from those in the non traumatic cases (Table V) Further, as will be shown in another section the sequence of local trauma and corresponding localized paralysis can be reproduced experimentally.





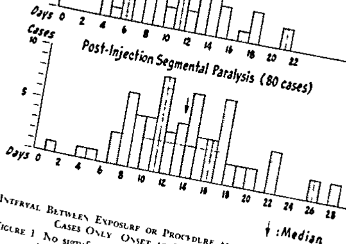


FIGURE 1. No significant difference in incubation periods is evident between traumatic and non-traumatic exposures

TABLE V

BULBAR POLIOMYELITIS IN POLIOMYELITIS CASES WITH AND WITHOUT RECENT TONSILLECTOMY TWO SERIES

	Poliomyelitis			
	Bulbar	*Other Forms*	*% Bulbar*	*P**
A 0–10 years				
Tonsillectomy	24	12	66.7	
No tonsillectomy	771	6063	11.6	001
B 3–7 years				
Tonsillectomy	12	4	75 0	
No tonsillectomy	96	395	19.5	.001

* From chi square of differences between bulbar and other forms
In both series P indicates that the probability of differences occurring by pure chance was less than 1 in 1000
Series A from Cunning, series B from Anderson; cited and tabulated by Faber, *et al*[63]

Neonatal Poliomyelitis and the Question of Transplacental Transmission of the Virus

The infrequency of fetal infection in human viral diseases in general has been commented upon by Goodpasture,[81] it occurs most often in smallpox and rare instances have also been reported in measles, chickenpox, equine encephalomyelitis and some other diseases. In poliomyelitis it appears to be even rarer. Abramson and his associates have recently reviewed 610 cases of poliomyelitis occurring during pregnancy and thirty cases in newborn infants without finding a proved case of fetal infection

In three reported instances, however, the occurrence may be regarded as a possibility In Kreibich and Wolf's[112] case, the mother had had poliomyelitis 3 weeks before the baby was born, and the latter died twelve hours after birth of asphyxia showing marked flaccidity of all the extremities. Post mortem examination showed some suggestive but not conclusive lesions (the microphotographs are dubious).

Johnson and Stimson[117] reported a case in which weakness of the abdominal muscles was detected at four days and the spinal fluid showed pleocytosis and increased protein. The mother had had symptoms suggestive of non-paralytic poliomyelitis six weeks before delivery. Tests of the infant's stool for virus were negative. This may be regarded as a possible instance of transplacental transmission but was regarded by the authors as unproved. More recently, Swarts and Kercher[169a] reported the case of an infant delivered by Cesarean section of a mother who, a few minutes before, had died of acute poliomyelitis. The infant's paralysis appeared on the 11th day. The virus was recovered from both mother and child. While the authors concede the possibility that the infant may have ingested virus containing maternal blood, this was probably a true case of transplacental transmission. However, it occurred under highly unusual circumstances which may have altered placental permeability.

In the case reported by Baskin *et al.*[7] the mother was in the acute stage which had begun four days before delivery. The infant showed a rise in temperature three and one-half days after birth but no symptoms until five days. The usual criterion for determining the end of the incubation period is the onset of symptoms, the usual time of onset of fever alone being unknown. Baskin deduced transplacental infection in this case from the shortness of the period after birth when fever began, assuming that the interval was less than the recognized incubation period of poliomyelitis. However, it is important to note that in John's[118] case of neonatal poliomyelitis, exposure was not from the mother but from the baby's brother who was in the acute stage of the disease and had handled the baby four hours after the latter's birth. In this case the baby developed *symptoms* at four days, or only a few hours more than the onset of fever in Baskin's case. In Frovig's case,[74]

also in which the mother was in the acute stage, the infant developed the first symptoms at four days.

Of thirteen reported cases of neonatal poliomyelitis[41] in which there was known exposure and in most of which the mother was in the acute and possibly viremic stage at the time of delivery, the average time of onset was 8.2 days after birth. It is highly probable in these instances that the mother was excreting virus in the feces, affording obvious opportunities for oral contamination of the baby in the birth canal or at the time of delivery.

The efficiency with which the placental barrier opposes passage of poliomyelitis virus from mother to fetus has been experimentally demonstrated by Weaver and Steiner.[199]

Summary

Poliomyelitis is a widely prevalent and readily communicable disease, acquired, commonly in asymptomatic form, by a large majority of the population in many areas during the first two decades of life. The low ratio of paralytic to non-paralytic cases points to the marked tendency of the infection to subside without producing serious clinical effects while inducing a high degree of immunity, usually permanent.

✓ Close contact with a carrier is usually required. The principal mode of infection appears to be by oral entry and not by inhalation, indicating fecal rather than airborne spread. This conclusion is based on epidemiological data corroborated by pathological and experimental observations. In a small minority of human cases, penetrating trauma initiates infection; particularly adenotonsillectomy and antigen injections. Despite the occurrence of viremia, there is no evidence that the disease is communicated by insect bites. Transplacental infection is extremely rare.

PATHOLOGY

FROM THE VARIOUS comprehensive pathological studies,[12,57,84,68,92,95,167] it is clear that neural lesions, together with the changes immediately referable to them, constitute the one constant feature of the pathology of poliomyelitis Their characteristic distribution in the CNS distinguishes poliomyelitis from other neurotropic viral diseases. So far as is known, only one other tissue, the LRE, displays lesions with great frequency[75,151,187] but these are not constant and, indeed are said to be rare in adult cases [181] Lesions in the heart muscle occur in about 32% of fatal human cases, according to Jungeblut's[119] compilation They are considerably less frequent in skeletal muscle, and are rare in liver, lungs, and other viscera So far as is known the epithelial surfaces, including those of the alimentary mucosa, show no lesions suggestive of viral invasion.

As first suggested by Charcot[25] *and later established by* many pathological studies, and with particular clarity by Bodian,[12] the primary reaction of the CNS to poliomyelitis virus consists of damage to the nerve cells, which is most conspicuous in the motoneurons but by no means confined to them. The earliest visible change is chromatolysis, with partial or complete disappearance of Nissl granules beginning around the nucleus, a process which in the milder grades of infection may be reversible and end in restitution of the integrity of the cell Cell necrosis (necrobiosis) in which the cytoplasm shows altered staining properties (acidophilic) or shrinks and becomes pyknotic, and the nucleus becomes dense and eccentrically placed, is also characteristic Necrosis, except in fulminant infections, is followed by neuronophagia in which the cell is attacked, broken up and eventually removed by mononuclear cells of mesodermal origin (microglia) and occasionally by

polymorphonuclear leucocytes.

Accompanying either chromatolysis or cell necrosis and neuronophagia there is usually an invasion of lymphocytes and microglia extending as a rule beyond the sites of neuronal damage, forming infiltrations of the interstitial tissues, and also dense perivascular infiltrates or "cuffs" in the spaces of Virchow-Robin which surround the venules and lead into the pia-arachnoid, thus forming patches of submeningeal exudate, and finally debouching into the subarachnoid space. The discharge of infiltrating cells through these channels is responsible for the pleocytosis and probably the excess of protein in the cerebrospinal fluid characteristically found in poliomyelitis, as well as in other forms of encephalomyelitis due to neurotropic viruses. Later, the inflammatory process subsides and leaves empty spaces (Zellausfall) to mark the sites of neurons that have been destroyed and removed.

The ectodermal glia (astrocytes and oligodendroglia) apparently is little altered during the acute stage of infection although during the stage of repair it undergoes active proliferation. The mesodermal glia (microglia, Hortega cells) on the other hand, accumulates markedly in loci of infection during the acute stage and as above noted, is a major factor in the process of neuronophagia. The microglial cells function similarly to the reticuloendothelial system which reacts conspicuously outside the CNS. It is probable, though not proved, that the former, like the latter, also phagocytizes virus, removing it from the CNS tissues and playing a part in preventing invasion of the neurons by blood borne virus.

In severe fulminant cases as first pointed out by Rissler, the lesions may be almost entirely confined to the nerve cells (chromatolysis necrosis) the secondary reactions (small cell infiltration, neu being mini-

mal or absent. A similar picture can be experimentally induced by cortisone in large doses.[103]

The distribution of the lesions in the nervous system in the human disease is essentially the same as that already described in the experimental infection, and the same areas show slight or no involvement. The constancy and severity of involvement of the reticular formation in the lower brainstem noted by Harbitz and Scheel and by Guizetti, among others, deserves emphasis.

Peripheral Ganglia

Here the pathological process is similar in nature to that in the CNS but somewhat modified by anatomical differences. Chromatolysis and cell necrosis occur but are often difficult to find and recognize with certainty because such changes commonly run a very rapid course and are so quickly and heavily overlaid by infiltrating and phagocytic cells (lymphocytes, microglia) and also by proliferation of the neurolemma cells of the capsule, that frequently only small neuronal cytoplasmic remnants, or none at all, can be found, and nerve cell loss can often only be inferred from the absence of recognizable neurons within an area of infiltration when compared with the distribution and numbers of nerve cells in the surrounding intact areas[58] (see Fig. 6). However, within such infiltrates, one can often recognize cell debris, or the outline of a capsule filled with infiltrating or proliferating cells.

Infiltrates, in some instances traceable to a single chromatolytic nerve cell, as in Figure 2, spread some distance beyond the initial focus through the interstitial tissue and occasionally are seen to meet a small blood or lymph vessel. Well defined perivascular cuffs are rare in the peripheral ganglia, probably because the lymphatics do not form well defined sleeve-like spaces around the blood vessels compar-



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(See facing page for legend.)

given low-power field. Moreover, the lesions were not uncommonly found in only a single segment of a ganglion. For this reason we have adopted the study of complete serial sections of each specimen as a necessary procedure for determining the presence or absence of lesions.



2

FIGURE 3 1. A lesion in a gasserian ganglion at sixty hours after feeding virus. A small infiltrative focus surrounds a chromatolytic nerve cell. C1086 ×440. 2. A lesion in a superior cervical sympathetic ganglion at fifty-six hours after feeding virus. The dense infiltration contains many microglial cells. In the center is a necrotic, pyknotic nerve cell on which a microglial cell impinges: early neuronophagia. C1072 ×440

The ganglia most often and severely involved in monkeys infected by swabbing[63] and feeding[64] have been the gasserian (V), the nodose (X), the superior cervical sympathetic, the celiac (sympathetic), and the petrosal (IX).

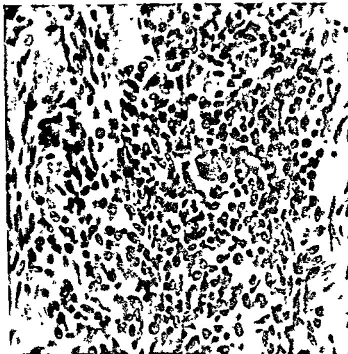
After infection has become established in the CNS, sec-



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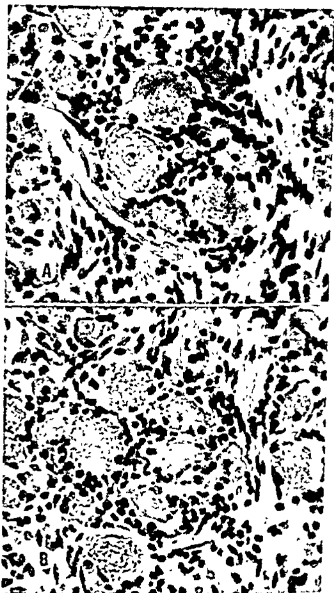


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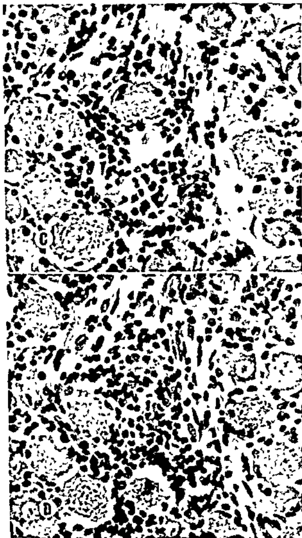
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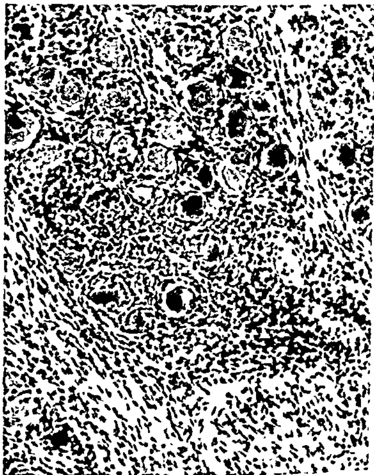
FIGURE 1. Lesion in a gasserian ganglion two days after oropharyngeal swabbing with virus; four successive sections. A, a cluster of nerve cells of which the lowermost is shrunken and pyknotic, the

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2

others normal B in the center of the cluster a chromatolytic cell has appeared which is also seen in C now partly surrounded by infiltrating cells D the neuron has disappeared and its site is occupied by *infiltrating cells C198 ×440*



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(See facing page for legend.)

ondary centrifugal spread to the peripheral ganglia occurs, producing lesions similar to those of centripetal origin. Thus, in human autopsy material as well as in the later stages of the experimental disease it is often difficult to say whether lesions in the ganglia have resulted from centripe-



2

FIGURE 5 Gasserian ganglia at fifty six hours after feeding virus 1 Within this focus six necrotic, pyknotic nerve cells and part of one chromatolytic neuron are visible Several normal neurons are present above the focus 2 In the center of this focus a circular arrangement of infiltrating cells and some nerve cell debris indicate neuronophagia A shrunken neuron is seen in the lower left portion of the focus The other nerve cells above and below the focus are normal C1117 ×220



FIGURE 6. Gasserian ganglion two days after simple feeding of virus. In this large, dense focus no nerve cells are visible, but comparison with the adjacent areas, in which many normal ones are seen, indicates that several have disappeared. Note the small paravascular infiltrate at the lower left edge of the focus. C1070. ×110.

tal ascent or centrifugal descent of virus.[87] However, when the nucleus in the CNS connected with fibers to a given ganglion is completely free of lesions it may be suspected

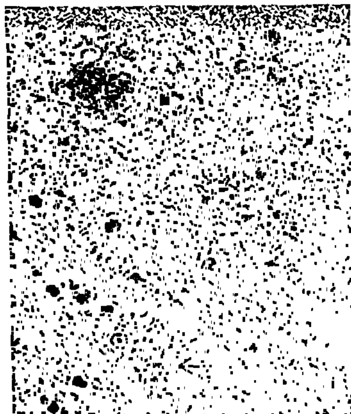


FIGURE 7 Gasserian ganglion at fifty-six hours after feeding virus There is a dense infiltrative focus within the ganglion at the upper left and a more diffuse one at the right Below the ganglion proper a lymphocytic infiltration of the nerve bundle is seen (arrow) CH17 ×110

that lesions in that ganglion are of centripetal origin In our human cases, this was frequently true of the trigeminal, vagal and superior cervical sympathetic systems [57]

In general we have obtained the impression, based

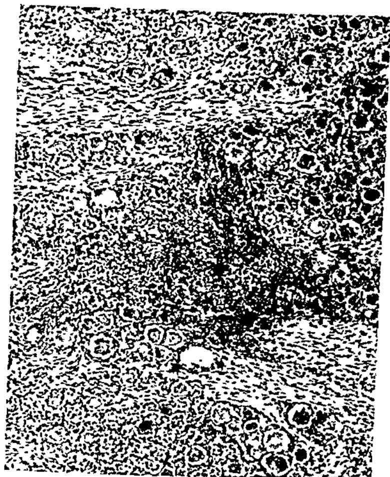


FIGURE 6 Gasserian ganglion two days after simple feeding of virus. In this large, dense focus no nerve cells are visible, but comparison with the adjacent areas, in which many normal ones are seen, indicates that several have disappeared. Note the small paravascular infiltrate at the lower left edge of the focus C1070. ×110

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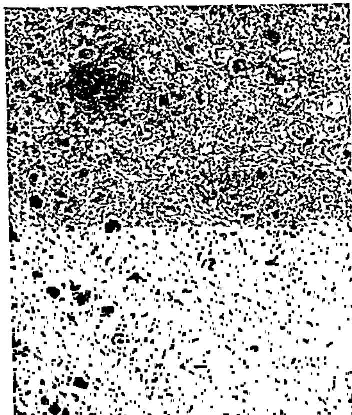


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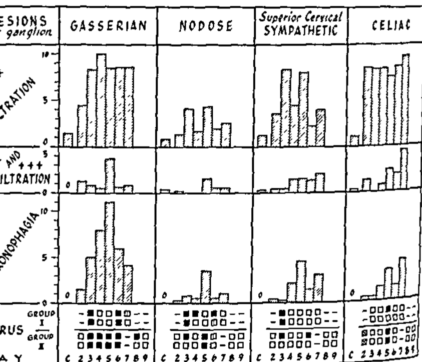


FIGURE 8 Lesions and virus recoveries in peripheral ganglia after atraumatic oropharyngeal swabbing, by days Note that virus was recovered mainly from the third to the sixth day, and only once thereafter, indicating a tendency for it to die out in the ganglia after a brief period of multiplication From *The Journal of Experimental Medicine*[64]

particularly on the early findings on the second and third days after exposure, that the initial lesions in the peripheral ganglia occurring before CNS invasion are more often due to entry of infection through single filaments of peripheral nerves (that is, derivatives of single neurons) than to entry by way of the blood stream

Inflammatory changes in peripheral nerve in poliomye-

litis have several times been reported. Thus Peabody, Draper and Dochez[169] in 1912 mention "cellular infiltration which is found along the nerve roots" in human cases, and Flexner and Amoss[79] speak of "the infiltrative process within the septa of the nerve roots" in the experimental disease in monkeys Also in infected monkeys, Nicolau and his associates[155] (1929) found interstitial infiltrations of nerves with lymphocytes and polymorphonuclear cells. Jordi[118] (1931) in seventeen of twenty infected monkeys found interstitial and small perivascular infiltrations in peripheral nerves, sometimes as early as twelve hours after the onset of paralysis Howe and Bodian[107] found perivascular cuffing and focal infiltrations in the mandibular division of the fifth nerve and also a questionable focus in the vagus We, too, have repeatedly found infiltrations in nerve bundles and trunks in or near infected ganglia (see Figure 7)

Degenerative changes like those following nerve section (Wallerian degeneration) are found in nerves, notably motor, whose neurons have been destroyed by the disease, but there is some evidence that nerve degeneration of another sort also occurs in poliomyelitis Thus, O'Leary and his associates[157] "found evidence to indicate that a difference exists between the changes occurring in fibers corresponding to affected segments of the cord in poliomyelitis and the course of degeneration following nerve section" Denst and Neubuerger[41] (1950), describing the nerve changes in acute human poliomyelitis, noted swelling, fragmentation and lost staining properties of axis cylinders and occasional interstitial lymphocytic infiltrations in the nerve trunks These authors, too, considered the lesions to be different from those of Wallerian degeneration Kausche,[122] studying the nerves in experimentally infected animals by electromicrography, found marked disintegra-

tive and disorganizing changes in the axons.

In view of the demonstration of the centrifugal spread of virus in the peripheral nerves, which will be discussed more fully in a subsequent section, and of the predominance of painful clinical symptoms in the disease from the onset, the histological changes in the peripheral nerves take on added significance

Extraneural Tissues

In 1949, Bodian,[12] who has exhaustively studied the histopathology of poliomyelitis, experimental and human, for many years, in discussing *extraneural tissues* stated "It is a remarkable fact that even in those non-nervous tissues from which the virus of poliomyelitis may be isolated at autopsy, its effect is so subtle that as yet it cannot be demonstrated by histologic means." So far as this statement implies that the presence of virus in extraneural tissues removed from subjects who have succumbed to the disease (that is, at the terminal stage of paralysis) indicates viral multiplication (vegetative phase) in those tissues, it may be seriously questioned, since multiplication of virus in a given tissue is generally manifested by lesions of the constituent cells, the reverse being unknown in poliomyelitis

There are alternative explanations to be considered for the presence of virus in extraneural tissues. it may be situated in the contained blood, it may be passively stored and accumulated as a result of removal from the blood by phagocytosis or it may be situated in the nerve fibers supplying the tissue as a result of centrifugal axonal spread. It will be presently shown that the last of these processes occurs to a remarkable extent by the time paralysis has supervened In my opinion, therefore, the demonstration of specific cellular lesions is mandatory before one can

conclude that any given tissue has been actively parasitized by poliomyelitis virus

Actually the passage just quoted disregards the not uncommon association of virus and lesions in the LRE and occasionally in some other tissues in human patients The discovery of viremia early in the experimental and in the human disease and the revived interest in the possibilities of extraneural infection as one stage in the development and evolution of the poliomyelitic process calls for a review of human histopathological findings and a general reconsideration of the evidence

Palpable enlargement of the lymph nodes and spleen is not a feature of the clinical picture of acute poliomyelitis However, in the human disease certain abnormal changes are frequently, though not constantly, seen postmortem in these structures The lesions have been particularly well described in the Rockefeller Monograph by Peabody, Draper and Dochez[160] (1912), by Burrows[22] (1931) by Landon and Smith[134] (1934) and more recently by Sommers, Wilson and Hartman[187] (1951) They have not been observed in the experimental disease

As described by Peabody and his associates from 11 human autopsies, the lesions may be summarized as acute swelling of the Peyer s patches (without change of the overlying mucosa), of the mesenteric nodes, to a somewhat less extent, of other nodes, and of the spleen, microscopically, the lymph nodules showed zones of lymphocytes surrounding pale centers consisting of large endothelial cells, similar to those found lining the lymph sinuses, these endothelial cells were markedly phagocytic, containing particles of necrotic cells, in the centers of the nodules were many broken-down cells and fragments of necrotic nuclei, most of which were lymphocytic, but endothelial cells also seemed to undergo swelling and disintegration; in the areas

with extensive necrosis there was often an invasion by polymorphonuclear leucocytes; in the lymph sinuses there was also extensive proliferation of endothelial cells with mitotic figures.

The description of the changes by Sommers, Wilson and Hartmann and found by them in 82% of their fifty human cases agree in the main with those just cited. They, too, noted as the principal finding degenerative, regenerative, and phagocytic changes in the reticulum, but, in disagreement with Peabody *et al*, stated that lymphoblasts and lymphocytes did not appear to participate in the process In five cases they noted intranuclear inclusions in the reticulum. Two cases showed acute myocarditis and two others, focal liver necrosis, the latter lesion was also described by Peabody and his associates. The changes in the lymphoid tissues were regarded by Sommers and his associates as morphologically similar to those in other virus diseases

It seems clear from these descriptions that both proliferation and degeneration occur mainly in the reticulum, which also displays phagocytosis Both the Rockefeller group and Sommers *et al* noted that the intestinal lymphoid tissue (Peyer's patches and mesenteric nodes) are somewhat more severely and frequently involved than those in other parts of the body According to Saphir,[181] lesions in the LRE are seen mainly in children and rarely in adults

The fact that the histopathological changes in the LRE have been reported almost exclusively in human tissues obtained postmortem when the disease is necessarily always far advanced, with much cell necrosis and inflammatory reaction in the CNS, has suggested to me[77] that they are secondary phenomena connected with defense and removal of damaged tissue, in which the formation of antibody and phagocytosis of circulating tissue detritus also participated

The discovery that viremia occurs early in the disease now raises the question whether direct viral action on the LRE follows the filtration, accumulation and phagocytosis of virus in it, and also whether virus might even multiply in these tissues to serve as a source of dissemination

Reasons have already been stated in the section on Properties of the Virus why viral multiplication in the LRE is highly improbable. The observed changes in it might be explained on the basis of active viral destruction, of formation of antibody and perhaps also of Selye's alarm reaction. In human material, virus has often been recovered from lymph nodes and tonsils,[314] even in patients without apparent poliomyelitis,[324] but with a curious lack of regularity and uniformity and with equally curious differences between the various regional nodes The data have been recently compiled by Wenner and Rabe,[211] who have added a number of personal observations. Positive tests for virus have been obtained most frequently in the axillary and inguinal nodes, least frequently in the cervical and mesenteric. Positive tests have also been frequently reported in the tonsils and pharyngeal mucosa, most recently by Sabin and Ward[114] (1/7 human cases) Since both tissues have usually been tested together it is difficult to state which one has contained the virus, in one of Sabin and Ward's[114] positive results, the tonsils were absent (though the possibility of residual pharyngeal lymphoid tissue would remain) Kempf and Soule[1] obtained questionably positive tests with spleen in two human cases

The explanation for these differences is obscure Since excretion of the virus into the alimentary lumen is one of the earliest features of poliomyelitic infection, one might entertain the notion that the regional nodes (tonsils, cervical nodes, Peyer's patches, solitary follicles and mesenteric nodes) take up virus at an early stage, form specific anti-

ANATOMICAL CONSIDERATIONS

THE EVOLUTION of poliomyelitic infection depends largely on the neurocytotropism of the virus, on its characteristic ability to spread through axons in either direction,[15,69] and on the efficiency of the barriers which stand between blood-borne virus and the neural parenchyma. The anatomy of both the peripheral and central nervous system[133,115,161,166] therefore merits special discussion.

If the virus penetrates the mucosa on which it is deposited and effects entry into the terminal nerve filaments therein—a process presently to be discussed in more detail —it is then free to follow a centripetal course along axonal pathways. The various peripheral pathways that may be involved in primary neural invasion are listed in the accompanying table, the trigeminal and vagal being perhaps the most important. Below the pharynx, the superficial nerve supply of the alimentary mucosa appears to be almost entirely vagal and sympathetic. Besides its afferent connections through the nodose ganglion, the vagus provides an efferent supply from the dorsal motor nucleus in the medulla to the submucous and myenteric plexuses *Nerve cells of the submucous plexus of Meissner* are present in very large numbers throughout the lower alimentary canal just underneath the mucosal surface and might therefore be accessible to virus in the lumen. While it is tempting to speculate that these cells might become primary foci of infection in the gastrointestinal walls, they offer such formidable difficulties to direct histological examination that they have been little studied and are not known to contain lesions in either the human or the experimental disease On the other hand, the celiac ganglion, which supplies *sympathetic fibers to the small and part of the large*

TABLE VI

PERIPHERAL NERVE SYSTEMS SUPPLYING THE ALIMENTARY AND RESPIRATORY MUCOUS MEMBRANES, AND THEIR CENTRAL CONNECTIONS

System	*Peripheral Ganglia*	*Region Supplied*	*Central Nervous System Connections*	
			Direct	*Secondary*
Trigeminal (V)	Gasserian (Semilunar)	a) Skin of face and scalp, b) Mucous membranes mouth, soft palate, nasopharynx	Pons main sensory, spinal V nuclei; Medulla spinal V; Cord (cervical) spinal V	Midbrain mesenceph V; Pons reticular formation, locus coer, Motor V, Motor VII, Medulla reticular formation
Gustatory VII special afferent, IX special afferent, X special afferent	Geniculate, Petrosal, Nodose	Taste buds tongue, palate, pharynx, epiglottis, larynx	Medulla nucleus intercalatus (?)	
Vagal (X) (a) General afferent	Nodose	a) Oropharynx, b) Tracheobronchial tree, c) Lower alimentary tract	Medulla nucleus of solitary tract	Medulla reticular formation, N ambiguus
(b) Efferent (autonomic)	Intrinsic bronchial, enteric (submucous, myenteric)	Bronchial tree, lower alimentary tract	Medulla dorsal motor nucleus of vagus	Medulla reticular formation
Sympathetic Superior cervical	Superior cervical	Vessels and glands of nose, mouth pharynx	Spinal cord intermediolateral columns $T_1 - T_3$	Brainstem reticular formation, hypothalamus
Gastrointestinal (a) Upper	celiac	Stomach small intestine, proximal colon	Spinal cord iml columns, $T_5 - L_1$	Same as above
(b) Lower	Inferior mesenteric	Distal colon sigmoid, rectum	Spinal cord iml columns, L_{1-4}	Same as above
Parasympathetic, sacral	Pelvic	Distal colon, sigmoid, rectum	Spinal cord $L_4 - S_4$	

intestine, frequently contains poliomyelitic lesions. It may be noted that the spinal ganglia have few if any direct connections with the mucosal surfaces but only with the *deeper, mesenteric layers of the gut (Vater-Pacinian receptors)*, and are probably involved only by centrifugal axonal spread.

The cell bodies of the peripheral ganglia, with the exception of the sympathetic system, have peripheral and central axons; the former ending in arborizations in the surface epithelium and the latter ending in the CNS, thus providing a continuous, mononeuronal line of communication for axonally invading virus from the body surfaces into the interior of the CNS. Here, synaptic connections permit extension into neurons of the second order which in turn connect synaptically with a wide variety of other neuronal centers and thus afford opportunities for an extremely wide distribution of infection. The cell bodies of peripheral sympathetic fibers are connected by synapses in the sympathetic ganglia themselves with neurons of the second order in the thoracic portion of the spinal cord

Within the ganglia there are cell-to-cell synapses of limited extent, and also direct connections *through protoplasmic* bridges between adjacent cells; thus infection introduced originally into a single cell finds an opportunity to spread to adjacent cells in the same ganglion; but such spread, in our observation, has rarely been extensive.

In its terminal arborization, the individual nerve fiber loses its myelin sheath, and subdivides into a number of telodendra which lie between and end on the surfaces of the epithelial cells. Whether they actually present "bare" endings on the surfaces is difficult as a *rule to* determine with certainty since, with silver staining, the most distal filaments seem to be too small to be resolved under visible light, indicating diameters of less than 0.2 micra.[44] Des-

quamation of epithelium, which is a continuous and fairly active process in the alimentary tract, must involve shedding of the superficial nerve filaments, a process which may well provide an anatomical basis for viral excretion.

Nearly all tissues of the body are abundantly supplied with end arborizations, hence, at stages of poliomyelitis in which centrifugal intraneural extension of virus has become widespread, it is virtually impossible to determine by subinoculation or culture whether or not the extraneural tissue elements themselves have participated in the viral multiplication [87]

Barriers Between the Blood and the Nerve Cells

The relationship of the so called "blood-brain barrier" to the pathogenesis of poliomyelitis was much discussed in earlier studies but, following the discovery of axonal transmission of the virus, its importance was largely discounted The recent recognition of viremia as a feature of the early phase of the disease has now necessitated reconsideration of the subject

Two anatomically different barriers exist against the passage of colloids and particulate matter from the blood stream into the nervous system, (1) the choroid plexuses, between the blood and the CSF, and (2) the tissues (vessel walls, glia and perineuronal capsules) between the blood and the nerve cells (parenchymal or blood-neuron barrier). The choroid plexuses appear to be highly efficient, since virus rarely if ever enters the CSF[125] except after neural infection has occurred[69] and then only exceptionally Moreover, when virus is placed, without trauma, on the pia arachnoid surface it fails to penetrate the neural parenchyma [28] The parenchymal barrier also appears to be quite effective under normal conditions against the passage of colloidal and particulate matter from the blood into the

nervous tissues, as shown by the failure of intravenously injected dyes such as Congo red[93] and trypan blue[93] to stain the parenchyma of the brain or cord under normal conditions.

In the CNS the nerve cells are surrounded by a dense investment of ectodermal glial cells and their processes (astrocytes, oligodendroglia) which present an apparent defense against the passage of particulate matter from the blood into contact with the neurons In addition, the parenchyma is abundantly provided with phagocytic microglia cells, of mesodermal origin, which migrate promptly into areas of injury and infection and thus are available to pick up virus particles that may have escaped from the blood stream into the tissues.

In the peripheral ganglia, the barrier is somewhat less elaborate; the neurons are surrounded by a single-layer capsule of cells derived from the neurilemma of the nerve fibers and microglia is also present, but there appears to be no ectodermal glia Some of the early lesions described under Pathology are probably reactions of the parenchymal barrier tissues to circulating virus

These rather elaborate anatomical barriers might appear to be of major importance in defending nerve cells against invasion by various blood-borne pathogens, standing in contrast to the almost complete lack of anatomical defenses against cell-to-cell axonal spread However, as will be shown under Viremia and Invasion of the CNS, their efficacy in poliomyelitis is distinctly limited.

Relation to the Lymphatic System

In the CNS there is an analogue of the lymphatic system which drains outward into the sleeve-like spaces of Virchow-Robin surrounding the venules and thence into the subarachnoid space where its contents are mingled with the

cerebrospinal fluid.[97] The latter is partly absorbed through the arachnoid villi (including the Pacchionian bodies) into the blood stream and partly into the perineural spaces of the peripheral nerves where it eventually joins the lymph-

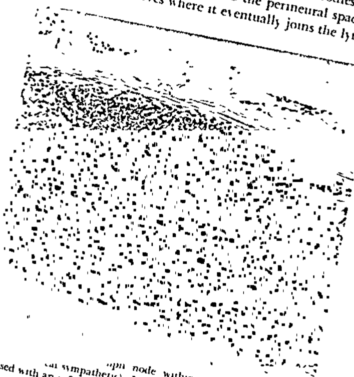


Fig ... (su... ...cal sympathetic) ...ph node within a peripheral ganglion ... This is a normal structure not to be confused with an infiltrative lesion Cynomolgus monkey (C500) ×40

atic system Only under exceptional and artificial conditions does flow occur in the reverse direction from the cerebrospinal fluid into the spaces of Virchow Robin ... Contrary to earlier belief poliomyelitis virus is therefore very unlikely to invade the CNS from the lymphatic system

Indeed, the virus rarely reaches the cerebrospinal fluid even after infection has become established in the CNS

In the peripheral ganglia the lymphatics are part of the extraneural lymphatic system, and drain into the regional lymph nodes, within the ganglia they parallel the blood vessels without surrounding them to form perivascular spaces like those in the CNS, hence, inflammation in the ganglia rarely produces marked "cuffing" such as is so frequently seen in the CNS.

Occasionally small lymphoid nodules are found at the margins of the peripheral ganglia and within their sheaths (Fig 9) They are readily distinguishable from pathological infiltrations

Bodian[11] has recently suggested that in poliomyelitis CNS invasion from the blood stream begins at the area postrema This is a small, highly vascular structure in the caudal medulla just under the ependymal surface of the fourth ventricle Wislocki and Putnam[212] observed that when Prussian blue reagents were injected intravenously this was the only area of the CNS that became stained. However, there was no staining of the adjacent ventricular walls, underlying tissues or adjacent nuclei, but some of the material was seen lying free in the ventricular lumen. They concluded that the area postrema serves as a source, additional to the choroid plexus, of cerebrospinal fluid. In our own histological studies of the CNS in poliomyelitis we only once found lesions in or directly traceable to the area postrema, nor so far as I know have such lesions been described by others. Apparently the nerve cells of this area, with specialized neurosecretory functions, described by Cammermeyer,[23] are not susceptible to poliomyelitis virus

The importance of oral entry makes it desirable to discuss briefly local conditions in the mouth, pharynx and intestine in relation to surface exposures

The oropharynx is covered with non-ciliated, stratified squamous epithelium over which a layer of rather tenacious mucus moves slowly, unaided by ciliary action, from traction by swallowing. Although the rate of movement in this region has not been accurately measured, it can easily be demonstrated by the simple method of clearing the throat and expectorating, that colored foods remain there for half an hour or more. Solid and semisolid foods tend to remain in part for considerable periods of time between the teeth, in the gingivobuccal folds, in the crypts of the tonsils, as well as in the pharynx. Bloomfield[10] placed charcoal paste in various regions of the mouth and pharynx and noted a backward flow converging on the base of the tongue; the material disappeared in fifteen to thirty minutes except in the tonsils where it remained over two hours and on a lymphoid nodule in the pharynx where it remained over an hour. The conditions in the mouth and pharynx, particularly the latter, therefore affords a considerable period of time for virus incorporated in food and mucus to make contact with the superficial tissues, an opportunity that is undoubtedly enhanced by friction from hard foods and the toothbrush.

In the intestine, the segmenting and pendular movements and the movements of the villi present highly favorable conditions for surface exposures and also for absorption of virus, apparently the latter is of greater importance (see section on Primary Invasion).

PRIMARY INVASION: PORTALS OF ENTRY

In nearly all cases of infectious disease the quantity of the specific pathogenic agent initiating infection is presumably quite small and multiplication must occur before pathological and clinical manifestations can emerge. In the case of viruses the pioneer extracellular invaders must find susceptible host-tissue for intracellular implantation and growth, and for each virus such tissues are usually limited to one or a few cell-types, whose consequent derangement determines the clinical and pathological characteristics of the disease.

For obvious reasons, the mode and localization (portal of entry) of primary invasion in poliomyelitis can best be elucidated by experimental investigation, relying mainly on the sites of the earliest lesions and, with certain reservations, of the earliest localizations of virus, after exposures reasonably comparable with those of human patients The comparability of conditions is extremely important For example, oral exposures of animals to heavy doses of virus are quite different quantitatively from initial human ("natural") contacts with the virus; they correspond much more closely with natural alimentary re-exposures during the secondary phase of viral excretion.

The neurocytotropism of poliomyelitis virus is not in dispute Recently the possibility has been revived[11, 155] that the virus may also have affinities *in vivo* for other cell-types Accordingly, two hypotheses of primary invasion are presently under debate 1) that the virus first parasitizes the cells of the "alimentary mucosa," which would include epithelial, lymphoid, collagenous and, from the esophagus downwards, neurocytic elements, 2) that the virus attaches itself to the terminal filaments of the superficial nerves of

the alimentary tract, ascends axonally to the nerve cells of the peripheral ganglia and first multiplies therein. A third hypothesis, not previously formulated, is that virus deposited on the mucous membranes is passively absorbed into the blood stream and then carried to nerve cells where it finds its locus of multiplication. These hypotheses will be separately considered.

THE QUESTION OF PRIMARY IMPLANTATION IN THE ALIMENTARY MUCOSA

While diarrhea and other signs of enteritis have occasionally been encountered in human patients, it is now generally accepted that these do not constitute an integral part of the symptomatology of poliomyelitis.[44] Lesions of the nasal, oral, pharyngeal, lower respiratory, and intestinal surfaces during the early stage of the disease have not been described, though during later or terminal stages destructive lesions of the lower alimentary tract have occasionally been observed, mainly in the bulbar cases, which are believed to be secondary to disturbances of the CNS.

Enlargement of the lymphoid apparatus—solitary follicles, Peyer's patches, mesenteric nodes—with both hyperplastic and degenerative changes have been noted by several observers, but these are not accompanied by inflammatory changes in the adjacent structures. Peabody, Draper and Dochez,[140] in discussing the lymphoid changes remark that "The mucosa over the Peyer's patches is, however, unaffected." Saphir[illegible] states that with the lymphoid alterations be found 'no evidence of inflammatory changes in the vicinity of the lymph follicles.' Sommers, Wilson and Hartmann,[197] in their comprehensive study of the intestinal lymphoid apparatus, make no note of such changes.

In the experimental disease, the histopathology of the alimentary mucosa has only rarely been studied, manifest

enteritis is unusual and when found is due to other causes Howe and Bodian[107] found no gastrointestinal lesions in a chimpanzee paralyzed after gavage exposure. In our many orally exposed monkeys gross changes have almost invariably been absent, as have microscopic ones in the few cases examined. No lesions have been found by us[54] in mesenteric nodes of monkeys sacrificed twelve, thirty-six and sixty hours after feeding virus in large quantities

Bodian has stated that "regular occurrence of virus in the oropharyngeal secretions and in the lower alimentary tract . . . has established these regions as the principal sites of virus multiplication." Besides the absence of lesions there are other valid grounds, I believe, for questioning the correctness of this view. If primary implantation and multiplication in the alimentary mucosa were the initial event in poliomyelitic infection induced by oral administration of the virus we should expect to find early, continuous and fairly constant appearance of the virus in the lumen, demonstrable in the stools. *Per contra,* in the absence of virus in the lumen, one should expect not to find other evidences of infection, including antibody formation

In a study of eighteen cynomolgus monkeys fed virus in fat-covered capsules to confine exposures to the intestinal tract, we[51] found that, apart from the immediate post-ingestional period (forty-eight hours), no virus appeared in the stools excepting in the one animal which developed paralysis and therefore had proved neural infection Since, as shown by post-ingestional recoveries of virus from the stools, the intestinal mucosa had been heavily bombarded, we obtained the impression that *the intestinal mucosa was not susceptible to primary viral implantation* Since then three rather elaborate sets of observations have appeared, one on chimpanzees and two on human subjects, in which virus was fed and the elimination of virus and the evolu-

TABLE VII

First Positive Tests for Virus in Stools of Chimpanzees After 59 First Feedings with Given Type of Virus (1,2)

From Howe, Bodian and Morgan[87]

Day	1-2	3-5	6-7	8-9	10-12	13-14	15-16	17-19	20-21	Total
Number animals	1	3	3	7	16	1	5	1	2	39*

* All tests negative in 20

tion of antibodies were carefully followed. In most of the chimpanzees and in all of the human subjects, no clinical symptoms of infection ensued.

In the study by Howe, Bodian and Morgan,[119] fifty chimpanzees were fed, sometimes repeatedly, with types 1 and 2 of virus in amounts ranging from forty thousand to six million intracerebral PD_{50} doses, the total number of first feedings with a given type being 59 (twelve with type 1 and eight with type 2). In twenty, or 34% in the stools were negative. In the remaining thirty-nine, the first positive tests, as shown in Table VII, occurred over the wide range of one to two to twenty to twenty-one days after feeding, with the peak at ten to twelve days. In seven instances, the first positive was obtained after one or more previously negative tests (Table VIII), occurring on the

TABLE VIII

Seven Cases in Which First Positive Test Occurred After One or More Negative Tests. Same Series as in Table VII

Day / Animal #	3-5	6-7	8-9	10-12	13-14	15-16	17-19	20-21	Type
B453			0	+				0	1
B465			0			+		0	1
C224		0	0			+	0		2
C226		0	0			+	0		2
C248		0		+	+				2
E766				0	+	0	0		1
E767				0	0	+		0	1

given ganglion. In some instances, the corresponding centers in the brainstem or cord were involved and in others, not, in the latter event, it could be presumed that the peripheral lesions were of centripetal origin; while in the former, they could be either centripetal or centrifugal.

These positive observations on human material therefore left open the possibility of neural entry of infection from the alimentary surfaces, a possibility which remained to be confirmed by experimental investigation. In place of a single site of primary penetration, as had been previously postulated for the olfactory mucosa, it was suggested that neural invasion could occur by any one of several different nerve systems connected with the alimentary tract, but appeared more frequent through the upper, oropharyngeal channels.

In a critical study of our paper and on the basis of their own observations, Bodian and Howe[16] correctly pointed out (apparently overlooking our own reservations) that some of the lesions might be centrifugal, that human material was not adequate for a full differentiation between centripetal and centrifugal spread (which we had ourselves stated) but concluded, in essential agreement with our own views, that entry in man apparently occurs through the V, VII, and X cranial nerve routes, and stated that fuller proof would have to come from experimental study. Investigations of ours bearing on this problem will be presented below Certain prefactory remarks on the conditions surrounding human infection are first in order.

The great majority of human poliomyelitic infections occur without preceding gross trauma and probably, as has been discussed, by the oral route It must be emphasized, however, that minor, frictional trauma in the mouth is a constant feature of daily life, as, for example, during mastication of hard foods, and the use of the toothbrush

It is interesting—although possibly not significant—that clinically evident poliomyelitis is rare before the age of one year when children begin to eat hard foods and to have their teeth brushed. Such minor trauma may well promote the surface penetration of virus from the oral and pharyngeal mucous membranes into underlying tissues. Further, a minority of human cases are traceable to gross trauma, notably tonsillectomy, and injections of antigens and probably some other materials. Experimental investigation of primary invasion must therefore employ methods analogous to these modes of exposure, which may be classified as: 1) atraumatic and mildly frictional, and 2) grossly traumatic.

ATRAUMATIC AND MILD FRICTIONAL EXPOSURES

In 1942 Miss Silverberg and I[6] had published a note concerning a cynomolgus monkey with previous zinc sulphate olfactory blockade which was exposed by spraying the mouth with virus (Per strain, type I) on three successive days. Two days after the last spraying the animal displayed some tremors and slight weakness of the arms and was immediately sacrificed in the belief that the symptoms were poliomyelitic in origin. However, on the basis of certain observations shortly afterwards in our own laboratory we are now inclined to believe that low-calcium tetany, due to a calcium deficient diet then in use, may have been responsible for the symptoms—for our purposes a fortunate error in diagnosis. A comprehensive histological examination of the peripheral ganglia and CNS was made, with the following results. Heavy lesions were found in both gasserian ganglia and both nodose (X nerve) ganglia. Moderately severe lesions were found in both petrosal (IX nerve) ganglia, in three of six cervical sympathetic

ganglia and two of ten upper thoracic ganglia. Small lesions, few in number, and probably not significant, were found in one geniculate (VII nerve) ganglion, in one lumber sympathetic ganglion and in two of fourteen thoracic spinal ganglia. In the medulla a few typical parenchymal and perivascular infiltrations, without definite cell necrosis, were found in and near the nucleus of the tractus solitarius but nowhere else. No lesions were found elsewhere in the brain stem, in the olfactory bulbs, the lower thoracic sympathetics, the celiac, the cervical or the lumbar spinal ganglia or in the spinal cord.

The distribution of the lesions in this experiment strongly suggested entry through the peripheral filaments of the trigeminal, sympathetic, and vagus afferent nerves in the mouth and pharynx with extension: 1) to the gasserian ganglion, 2) to the cervical sympathetic ganglia, and 3) to the nodose (vagus) ganglion and thence to the afferent vagus center (solitary nucleus) in the medulla, the only area of the CNS involved in this particular instance. The animal had fortunately been sacrificed at the earliest stage of its CNS involvement, before infection had had time to spread beyond the point of initial entry in the medulla.

Subsequent and more extensive experiments[55] were published by us in 1951 and 1953, on the results of atraumatic swabbing and of simple feeding of virus.

With the Wis'45 strain of virus (type 1) in a suspension of PD_{50} 4.9, eighty-one cynomolgus monkeys were exposed under nembutal anesthesia, as follows: cotton swabs dipped in the virus suspension were gently rolled (actually not rubbed) over the buccal and oropharyngeal surfaces for one fourth to one-half minute at intervals of about five minutes over a total period of two hours. The total amount of virus used in this experiment was 21,000 PD_{50} per ani-

mal, but since the animals were under anesthesia and in side-lying position, most of it drained out of the mouth and relatively little was swallowed, a difference of some importance from the subsequent simple feeding experiments The procedure was essentially atraumatic The exposed animals were divided into two groups, one being used for histological examination of the gasserian (V), nodose (X), superior cervical sympathetic and celiac ganglia, and CNS, the second for subinoculations of the same ganglia (in pools of like ganglia from two to four animals each). Each group was subdivided according to days after exposure when sacrificed, the histological series from the second to the seventh day, inclusive, and the subinoculation series from the second to the ninth, inclusive It was therefore possible to compare the chronological relations between the evolution of the pathological changes and the appearance of virus in like ganglia Thirteen animals were kept as survival controls, of which ten developed paralysis seven to sixteen days after swabbing Nine un exposed monkeys were sacrificed as histological controls The results of the observations are condensed in Table X.

It will be noted that none of the control animals showed any neuronophagic or chromatolytic lesions in the ganglia. In the test animals the gasserian ganglia, which furnished the main nerve supply to the exposed areas in the oral cavity, were most heavily and regularly involved Here chromatolytic and neuronophagic lesions appeared in moderate numbers at two days after exposure (see Figures 2 and 4) and later rapidly increased in numbers and intensity, reaching a peak on the fifth day, after which they declined Virus was first detected, by subinoculation, on the third day and was found on each succeeding day including the eighth, but not on the ninth day

In the nodose ganglion which supplies the pharynx

TABLE X

LESIONS AND VIRUS RECOVERIES IN PERIPHERAL GANGLIA AFTER OROPHARYNGEAL SWABBING

Ganglia	Controls	Days After Exposure 2	3	4	5	6	7	8	9	Totals
Gasserian										
Nph	0/18	5/8	4/6	8/8	8/8	7/8	6/8	—	—	38/46 (83%)
Virus	—	0/2	4/4	2/4	2/4	4/4	1/2	1/2	0/2	14/24 (58%)
Nodose										
Nph	0/18	1/8	2/6	2/8	5/8	3/8	4/8	—	—	17/46 (37%)
Virus*	—	0/2	3/4	1/4	2/4	3/4	0/2	0/2	0/2	9/24 (37%)
Sup cerv Symp										
Nph	0/17	2/8	1/6	4/7	7/8	3/8	7/8	—	—	24/45 (53%)
Virus	—	0/2	3/4	0/4	0/4	1/4	0/2	0/2	0/2	4/24 (17%)
Celiac										
Nph	0/7	1/4	1/3	1/4	3/4	2/4	3/4	—	—	11/23 (48%)
Virus	—	(U/2)	0/4	0/4	3/5	0/4	0/2	0/2	0/2	3/22 (14%)

Nph neuronophagia *Numerators* number of ganglia positive, or number of animals positive for virus *Denominators* number of ganglia examined, or number of animals subinoculated

* In part of the tests for virus, the nodose and petrosal ganglia were combined U unsatisfactory tests

and gastrointestinal tract, and the superior cervical sympathetic and celiac ganglia supplying, respectively, the upper and lower portions of the alimentary tracts, neuronophagic and chromatolytic lesions were found in smaller numbers, also beginning at two days and increasing later but not to the height shown by the gasserians. From the nodose and cervical sympathetic ganglia virus was recovered on the third day and irregularly thereafter but not after the sixth, and from the celiac it was only recovered on the fifth day.

These experiments proved that the virus of poliomyelitis when placed with minimal friction on the oral and oropharyngeal surfaces reaches the regional ganglia in *less*

than forty-eight hours (since lesions were already present at that time) and most regularly and intensively in those ganglia corresponding with the site of exposure. The briefness of the interval between exposure and the appearance of lesions is indicative of direct passage along neuronal axonal pathways from surface to nerve cell Observations on the CNS will be described presently

To determine whether simple feeding, a more "natural" method of exposure, would give comparable results, another series of experiments[66] was performed In these eleven cynomolgus monkeys with previous section of the olfactory tracts were fed eighty thousand PD_{50} of the same strain of virus mixed with their ordinary food, all of which was eaten and swallowed Three animals were sacrificed at three days, four at five days and four at six days Pooled like ganglia from each day were tested for virus, and parallel tests for viremia were made. Comprehensive histological examinations of the CNS of all eleven animals were made, which will be described in a later section Virus was recovered from the nodose (vagal) ganglia on the third day and from the gasserian, nodose, superior cervical sympathetic and celiac ganglia on the fifth and sixth days (no subinoculations were made at four days) In a later study,[47a] histological studies of ganglia were made at two and one-half and three days after simple feeding Typical lesions were found in the gasserian, nodose and superior cervical sympathetic, which were heaviest and most constant in the gasserian (see Figs 3, 5, 6 and 7) In one of the latter, with particularly heavy involvement, the infiltrations were heaviest on the peripheral side of the ganglion and in the adjacent nerve trunks while the central side and the sensory root showed little or no involvement In this instance the evidence of centripetal ascent of infection was particularly strong.

The results from feeding were therefore *in essential* agreement with the experiments on exposure by *swabbing* In addition, tests for viremia were positive on the *third*, fifth and sixth days, which will be discussed in the section on Viremia.

SUMMARY

Experimental proof has been obtained that poliomyelitis *virus is capable* of effecting primary invasion from the *oropharyngeal* surfaces through axonal channels into the *neurons* of the regional peripheral ganglia under *conditions involving* minimal surface friction or none at all

THE QUESTION OF DIRECT ENTRY BY ABSORPTION INTO THE BLOOD STREAM

This process is demonstrable when large amounts of virus are fed to cynomolgus monkeys. Virus has been observed in the blood by us as early as thirty-six hours after oral inoculation, and by Horstmann[102] as early as twenty-four hours A serious error of interpretation could easily be made with such experiments if one failed to take into account the extreme quantitative dissimilarity of the experimental conditions from those of natural primary exposure, in which only the most minute amounts of virus enter the alimentary tract Investigating the effects of feeding small amounts of virus (500 PD_{50}), we[54] found that viremia did not begin until five days later, the time at which, as shown by other studies, the ganglia were already infected by neural entry, and excretion had begun Moreover, for reasons to be discussed more fully under Viremia, ingested virus reaching the lower alimentary tract and absorbed is promptly taken up by the LRE (liver, mesenteric nodes) It appears probable that only when very large

amounts of virus are present does enough escape this process of sequestration to reach the blood stream, a condition that occurs naturally only after excretion has begun

It seems likely, therefore, that direct *primary* entry into the blood stream, although theoretically possible, must be very exceptional If it occurs at all, it must be minimal and unlikely to pass the blood-neuron barrier.

INTRODUCTION BY GROSS TRAUMA

Reference has already been made to post-traumatic human poliomyelitis, and its characteristic localization of paralysis related to the site of trauma. Experimental investigations have confirmed this relationship Sabin's[175] early experiments, in which he used the MV (type 2) strain and rhesus monkeys, showed that deep inoculations of virus in the tonsillopharyngeal region consistently resulted in bulbar paralysis In one animal this also followed tonsillectomy after preliminary application of virus to the tonsillar surface. Our subsequent investigations were more uniformly successful when the latter method, cynomolgus monkeys and the Wis'15 (type I) strain were employed In addition, the effect of tonsillectomy on animals previously infected by intrathalamic inoculation and the preventive effect of 2% tincture of iodine applied to the throat after virus swabbing were also investigated The results are shown in the accompanying table

Tonsillectomy performed on uninfected animals with virus on the tonsillopharyngeal mucosa was thus shown to produce the typical picture of bulbar paralysis seen in human cases, with an incubation period corresponding to that of animals inoculated by other routes In animals with infection already established in the CNS the incubation period was shorter and the paralysis more extensive It was suggested that the results of this method corresponded

TABLE XI

TONSILLECTOMY AFTER PHARYNGEAL SWABBING AND AFTER INTRATHALAMIC INOCULATION

	Pharyngeal Swabbing		*Intrathalamic Inoculation*		
	Tonsillectomy Group I (No Iodine)	*Group II (2% Iodine)*	*Controls (No Tonsillectomy)*	*Group III Tonsillectomy)*	*Controls (No Tonsillectomy)*
Animals exposed	7	6	7	6	75
Poliomyelitis	7	0	5	6	75
Onset (days)	7–10	—	8–10	5 (2)*	av 8 7
Bulbar paralysis	7	—	1	5**	13
Spinal paralysis	3	—	4***	5	62
Cervical	3	—	4	5	
Thoracic	0	—	?****	5	?****
Lumbar	0	—	?	5	?

* 5 days after inoculation 2 days after tonsillectomy

** 1 animal found dead, not included in list of paralyses, had typical lesions

*** 1 animal, not included here, had typical lesions in the cord.

**** Not specifically noted or analyzed

Controls left column, animals inoculated at same time as test series; right column, animals inoculated at other times with same strain of virus and developing paralysis

with the rare human cases in which the disease begins three days or less after tonsillectomy The prompt virucidal effect of the mild tincture of iodine applied to throat was also demonstrated

A third, and perhaps more important source of virus for traumatic entry into regional nerves and the production of post-tonsillectomy bulbar paralysis may be the tonsillar tissue itself. Supporting evidence for this view is to be found in the observations of Kessel and Moore[124] and the experiments of Sabin[163] Testing the tonsils of 136 non-poliomyelitic patients without discoverable contact with the disease, Kessel and Moore obtained positive results with three of the forty-nine pools. The correspond-

ing pools of stools were also positive. The positive speci-
mens were obtained just before the 1912 epidemic in Los
Angeles While none of these patients developed clinically
apparent poliomyelitis, the study showed that the tonsils
can harbor the virus in the course of subclinical infection
and thus provide a potential hazard under conditions of
local trauma The seriousness of this hazard is revealed
by Sabin's experiments on intratonsillar injections men-
tioned above Obviously, local preoperative antisepsis could
have no preventive value against this mode of entry

In regard to the effect of injections on poliomyelitis, the
homologous localization of paralysis following introduction
of virus into peripheral nerves has been frequently demon-
strated, first by Landsteiner and Levaditi[135] in 1909, who
stated that "the virus is capable of propagating itself along
the nerves and thus can reach the central nervous system"
A similar result in human subjects was observed after sub-
cutaneous injections of an incompletely inactivated vaccine
containing MV virus.[136]

Following the observations of Martin,[144] McCloskey,[145]
and others that injections, principally of immunizing
agents and particularly those containing pertussis vac-
cine, occasionally resulted in homologous poliomyelitic
paralyses, the subject became of great practical importance
It raised three main questions 1) do such cases result from
primary introduction of virus contaminating the skin or
needle?, 2) do they result from localization in already in-
fected individuals of circulating virus in the traumatized
tissues?, and 3) does injected material (toxoid, vaccine, etc)
itself promote viral infection? Figure 1 in which the inter
vals between injections and tonsillectomies, respectively,
are compared with those after single, non-traumatic ex-
posures indicates that in the great majority of instances of
post-injectional and post-tonsillectomy poliomyelitis the

Excretion began in our experiments as early as two days after infection was established in neuronal centers, and continued up to and including the day of onset of symptoms (later periods were not studied).

Since in the swabbing experiments previously described typical lesions in the ganglia were observed as early as two

TABLE XIV

EXCRETION OF VIRUS IN NASOPHARYNGEAL SECRETIONS AND STOOLS AFTER VARIOUS PARENTERAL EXPOSURES

Procedure	*Number of Experiments (Animals)*	*Positive Tests for Virus by Days* 1	2	3	4	5	6	7	8	9
Infraorbital nerve dip	7 (28)									
Np washings		—	1/7	2/7	4/6	—	—	—	—	—
Stools		—	2/6	1/7	4/6	—	—	—	—	—
Inoculation in gasserian ganglia	4 (16)									
Np washings		0/1	0/3	0/3	2/3	—	—	—	—	—
Stools		—	0/2	0/2	1/3	—	—	—	—	—
Inoculation in celiac ganglion	3 (12)									
Stools		0/2	0/2	0/2	1/2	1/2	1/3	1/2	1/2	1/2
Intrathalamic inoculation	3 (12)									
Np washings		—	0/2	0/2	1/3	1/2	0/1	—	—	—
Stools	1 (4)	—	0/2	0/2	0/2	0/1	—	—	—	—
Intravenous inoculation										
Np washings		0/1	0/1	—	—	—	—	—	—	—
Stools		0/1	0/1	—	—	—	—	—	—	—

Denominator number of experiments with tests, *numerator*· number of positive experiments

days after surface exposures and in the parenterally inoculated animals excretion began as early as two days after initiation of infection in the ganglia, it was inferred that excretion might begin four to five days after surface exposures This time interval was corroborated by subsequent experiments[67] in which throat washings obtained four days

after oropharyngeal swabbing gave positive tests in three of six cynomolgus monkeys.

Some experimental data[32] have been obtained on the pathways by which virus is excreted Intravenous injections of twenty-four to forty thousand PD_{50} of the same strain of virus were made into each of four cynomolgus monkeys;

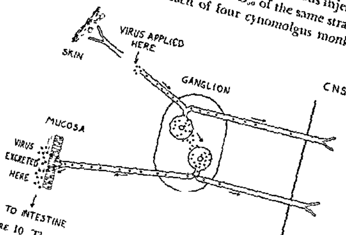


FIGURE 10 The diagram illustrates the fact, demonstrated by the experiments cited, that excretion of virus into the alimentary lumen results from centripetal followed by centrifugal nerve-borne spread to and from the peripheral ganglia, and is independent of initial exposure and infection of the alimentary mucosa itself

the nasopharyngeal washings were collected at two to four hours and twenty-four to twenty-seven hours, and the stools at zero to twenty-four hours, and twenty-four to forty-eight hours, subsequently All subinoculations of these materials failed to reveal the presence of virus. Viremia (which was massive in this experiment) therefore does not by itself appear to account for excretion The centrifugal passage of virus through peripheral nerve and

its relation to excretion were studied in another series of experiments, which will be more fully described in another section. It was shown that after sciatic nerve dip and after intrathalamic inoculation, the virus spreads centrifugally in peripheral nerves and when it has reached their distal segments, excretion occurs both into pharynx and intestine. Excretion did not consistently parallel viremia in these experiments.

Excretion by way of the lymphatics is highly improbable since these normally drain away from the surface into regional lymphnodes and thence into the main lymph ducts and blood stream. It is of interest to note that Yoffey and Drinker[214] were unable to detect poliomyelitis virus in the lymph ducts of intracerebrally infected monkeys.

It seems reasonable to conclude that the excretion of virus results from preceding infection of neuronal centers (peripheral ganglia, CNS) followed by centrifugal passage through peripheral nerves to the surface.

The reason for the long continued excretion of virus in human patients, which may last several weeks, is not clear. Since viremia and extension of the disease process subside at about the time when immune antibodies appear in the blood in measurable amounts,[11] it may be inferred that the source of the excreted virus after this time must be tissues not accessible to circulating antibodies in effective concentration. These might be perikarya of viable neurons continuing to harbor virus in sublethal quantities, or even axons still containing virus and propagating it centrifugally after recovery of the cell body. The subject requires further investigation.

Excretion of virus into the alimentary lumen, which begins so early and continues so long, must inevitably renew and cumulatively increase the chances of exposure and invasion, since it may be assumed that during the

early phases of infection the amounts of virus excreted become progressively larger as multiplication proceeds (see Fig. 12). Some direct evidence of reinvasion was obtained in our experiments[44] on unilateral nerve dip when in addition to the regular recoveries of virus from the ipsilateral ganglia, virus was also recovered in two instances on the fourth day from the contralateral ganglia, and on the same or a previous day from the pharyngeal washings and stools.

Reinvasion presumably continues—principally during the incubation period—until the defensive mechanisms, humoral and cellular, come into effective play. Up to this time, new foci of infection are formed which not only increase excretion but may serve as potential sources of viremia. It deserves to be emphasized that *reinvasion is a major factor in the evolution of the infective process in poliomyelitis.*

Summary

The excretion of poliomyelitis virus is traceable to infection of neurons in the peripheral and central nervous systems, followed by centrifugal spread through peripheral nerves to the surface. Excretion may commence as early as four to five days after initial exposure. It is not due to multiplication in the extraneural elements of the alimentary mucosa, nor to viremia. *Excretion leads to an expanding cycle of reinvasion and re-excretion.* Invasion continues until immunity comes into effective play. Excretion lasts much longer, presumably because foci of infection remain that are inaccessible to circulating antibody, these may be peripheral axons.

VIREMIA

UNTIL recently, poliomyelitis virus had occasionally been recovered from the blood of infected animals,[26,74,147] but with two exceptions not from human patients. The most extensive human studies were those of Ward, Horstmann and Melnick[196] in which blood samples from 111 patients were tested with only one positive result, in a non-paralytic case. Koprowski, Norton and McDermott[128] obtained the only other positive test by a series of blind passages through mice, this was with blood taken three days after onset from a non-paralytic patient The apparent rarity of viremia was accepted for several years as proof that it played no part in the pathogenesis of the disease.

However, investigations published in 1952 showed that viremia occurred with some regularity in orally exposed animals during the presymptomatic stage, and subsequent studies showed that it also occurs in human patients during the early stages Thus, Horstmann[100] announced the recovery of virus from the blood in seven of ten cynomolgus monkeys and in three of four chimpanzees four to six days, but not earlier or later, after ingestion of virus; the recoveries being three to seven days before the appearance of paralysis These observations were supplemented by Bodian[11] who obtained virus from the *serum* of three of four chimpanzees eight to fifteen days after feeding and added the important observation that virus disappeared from the blood at the time specific antibodies appeared. More recently, Horstmann and McCollum[103] obtained positive tests from human blood by tissue culture *and simultaneously from throat and rectal swabs,* of four children in one family, three of whom showed symptoms at the time (fever, headache, anorexia, vomiting) and one of whom was asymptomatic

None of the children developed paralysis The amounts of circulating virus were probably small (as was evidently true of the case reported by Koprowski *et al*), since no positive tests were obtained by direct intracerebral inoculation into monkeys Bodian and Paffenbarger[17] have also recently recovered virus from the blood of three children with fever who failed to develop paralysis

Obviously the conclusion drawn in 1946 by Ward, Horstmann and Melnick[196] from their many negative tests "that the presence of poliomyelitis virus in the bloodstream appears to be neither a common event nor a necessary factor in the pathogenesis of the human disease" must now be modified However, the mechanism or mechanisms responsible for viremia and the part that blood-borne virus plays in the disease process call for further investigation

We[47] have conducted several feeding experiments with massive dosage of eighty thousand PD_{50} per animal, one of which yielded positive tests for viremia at thirty-six hours, but none at twelve and eighteen hours. In one series, the liver gave a positive test at thirty six hours while systemic blood removed at the same time was negative This suggested that the delayed appearance of viremia may be due to the fact that intestinally absorbed virus is held back temporarily by the macrophages of the liver and released into the general circulation only when the latter are overloaded In Horstmann's[102] later feeding experiments, also with massive dosages, viremia appeared at twenty-four hours, once in a cynomolgus monkey and once in a chimpanzee Her observation that after twenty-four hours viremia disappeared for a few days and then recurred is interesting and, I believe, highly significant

The twenty-four to thirty-six hour interval between oral exposure and the onset of viremia seems much too short a period for intracellular invasion and multiplication of

the virus and its release into the general circulation in detectable amounts to occur. The large dosage of virus and the time relationships in the experiments just cited stand in favor of the view that the initial viremia resulted from direct, passive absorption similar to that known to occur with undigested protein[142,193] through the intestinal mucosa into the portal and then the systemic circulation as a sequence of *massive* surface exposures. Such heavy exposures could hardly occur under natural conditions at the time of primary entry, but would be reproduced after virus has multiplied within the body and been excreted into the alimentary tract. Horstmann's[103] recent experiments can be interpreted as showing both processes: initial viremia from passive absorption and, after an aviremic period, secondary viremia from active infection, excretion and reabsorption. It is significant that in her study of human patients, viremia was accompanied by virus in the throat and intestine.

For further investigation of the hypothesis just outlined, another series of feeding experiments[47] was performed, this time using small amounts of virus as a closer approach to natural primary exposure. Five hundred PD_{50} of virus were fed to each of four cynomolgus monkeys and samples of blood were removed from each at two, four, five and six days, pooled by days and tested by subinoculation. Samples of stool were also collected, pooled by days and tested by subinoculation. Of the original animals, two became paralyzed at nine and eleven days, one died without observed paralysis at thirteen days, and one remained well. Virus was recovered from the stools on each of the five days following ingestion, thus obscuring the transition from passive to active excretion, but viremia did not occur until the fifth day and was still present on the sixth. The almost immediate appearance of viremia after feed-

ing large amounts of virus when compared with its delayed onset (five days) after feeding small amounts, indicates that *viremia occurs when virus is abundant in the intestinal lumen, a condition presented artificially by massive ingestion and naturally by massive excretion* Both of these conditions were demonstrated by Horstmann's experiments cited in the last paragraph. It will be noted that in our experiments the onset of viremia was at approximately the same five-day interval as we had previously estimated to separate initial surface exposures and the commencement of excretion (see section on Excretion).

A second source of viremia is infection of the nervous system In our experiments[67] on centrifugal spread and also Horstmann's[102] experiments, viremia was detected in cynomolgus monkeys as early as four days after intracerebral inoculation In two of our animals it was present six days after inoculation, while at the same time virus was not present in the stools or pharyngeal washings

The relationships of viremia to invasion of the CNS will be discussed in a later section In regard to the peripheral ganglia, it was found as early as 1914 by Flexner and Amoss[10] that virus can be recovered from them after intravenous inoculation, an observation which we have confirmed The question remains, however, whether this indicates actual infection of the neurons, or merely the passive presence of virus in blood or phagocytic cells within the ganglia This question can only be answered by determining whether or not significant lesions are present at the same time.

In a preliminary study we[95] injected intravenously small (100 PD_{50}) amounts of the Wis'45 strain in a series of eight cynomolgus monkeys, sacrificing four, two days, and four, three days later. At two days, a paralytic take was obtained with the pooled superior cervical sympathetic ganglia and

a questionably positive inapparent take with the celiacs At three days, an inapparent take, also dubious, was obtained from the gasserians No histological examinations were made in this study of intravenously inoculated animals

Subsequently, another experiment[47] was performed in

TABLE XV

VIRUS RECOVERY FROM PERIPHERAL GANGLIA AFTER INTRAVENOUS INOCULATION

	Series 1	*Series 2*
PD_{50}	*100*	*6000*
Subinoculations		
At 2 days	0, 0	P
Gasserian	0, 0	P
Nodose	0, 0	P
Sup cerv symp	0, P	P
Celiac	0, NP?	—
At 3 days		
Gasserian	0, NP?	P
Nodose	0, 0	P
Sup cerv symp	0, 0	0, 0
Celiac	0, 0	—
At 5 days		
Gasserian	—	0, 0
Nodose	—	0, 0
Sup cerv symp	—	0, 0

P paralytic take NP? non-paralytic, questionable take, infiltrative lesions of limited extent 0 negative — no test Wis'45 strain of virus

which large doses (six thousand PD_{50}) of the Wis'45 strain were inoculated intravenously Here virus was recovered by subinoculation from the gasserian, nodose and superior cervical sympathetic ganglia at forty-eight hours, and from the first two of these at seventy-two hours, but from none of them at five days (Table XV), results which strongly suggest that the positive tests were due to virus in the contained

blood and that actual infection of the ganglia had not occurred. The histological controls supported this view. Serial sections of the same ganglia from animals intravenously inoculated with the same strain and doses of virus and sacrificed at forty-eight and seventy-two hours, showed only rare minimal lesions, without neuronophagia, such as we have commonly found in normal animals and regard as having no pathognomonic significance. Spinal ganglia were also examined. Of these the lumbar, cervical and most of the thoracic showed no significant lesions. In a few of the thoracic ganglia some infiltrations of moderate size were found but no signs of neuronal damage. As a whole the findings, coupled with those of pharyngeal swabbing and simple feeding described under Portals of Entry, show that viremia is much less likely to produce infection of the peripheral ganglia than is direct nerve-borne entry.

The discovery of viremia in poliomyelitis has been heralded by certain students of the disease, notably Paul[158] and Bodian,[11] as furnishing evidence for the existence of a primary extraneural, "systemic" or "vascular" phase of the infection, in preference to the view that viremia is a secondary or incidental phenomenon. On this basis Paul postulates a "new concept," and an "entirely new philosophy" of the disease which, however, is actually a reversion to the view formulated by Peabody, Draper and Dochez[160] in 1912 and even more explicitly by Draper[illegible] in 1917. In a review published in 1933 I[17] brought out some of the inconsistencies and difficulties in accepting this view and the fact that the clinical features are explained better on the basis of a purely neural infection.

In another section of the present paper I have again presented this interpretation of the clinical manifestations. Paul apparently still regards the "minor illness" (i.e., the symptoms and signs of mild, non-paralytic cases, of the first

phase of the diphasic cases and of the preparalytic phase of paralytic cases—all of which are similar) as representing a "systemic" or non-neural syndrome, preceding and independent of invasion of the CNS. The term "systemic" is a vague one, much used by clinicians to indicate a lack of localization, with connotations of "toxicity," and has no exact pathological counterpart.

It would seem hardly necessary to point out that viremia by itself is incapable of producing symptoms, since circulating virus is almost certainly extracellular (both Bodian[11] and Horstmann[102] have recovered it from the serum) and, so far as is known or probable, it does not produce a toxin. No lesions have been demonstrated in the extraneural tissues, even the lymphoid, during the initial stages of the infection Hence, the referral of early symptoms to extraneural sources as implied by the terms "systemic" and "general" infection is quite unsubstantiated by objective evidence On the contrary, as will be shown under Clinical Manifestations, nearly all of the characteristic onset symptoms of poliomyelitis are specifically neural.

Summary

Experimental studies show that viremia can be produced in two ways 1) by passage of virus through the intestinal mucosa into the blood stream, probably via the LRE or the portal circulation and liver, from which, after a brief delay, it reaches the systemic circuit (Fig. 3), and 2) by "overflow" from foci of infection in the nervous system Oral administration of large amounts of virus to apes, which is followed by viremia within twenty-four to thirty-six hours, is not comparable to the initial stage of natural exposure wherein only minute amounts of virus are engaged, but are comparable with conditions obtaining

during the secondary stage of viral excretion after infection has been established in the nervous system. When minimal amounts of virus are fed to animals, thus paralleling the conditions of initial human exposure, viremia begins at five days, apparently resulting from primary infection of inner, neural foci followed by excretion into the alimentary tract and passage into the blood stream

It is highly improbable that viremia by itself causes clinical symptoms

INVASION OF THE CENTRAL NERVOUS SYSTEM

DESPITE decades of research into the problem divergent views are still held as to the mode or modes by which poliomyelitis virus customarily invades the CNS However, the probabilities have now narrowed down to two: 1) axonal ascent through infected peripheral nerves, and 2) invasion from the blood stream during the phase of viremia. Ascent through the lymphatics into the CSF and thence into the substance of the CNS can safely be regarded on anatomical and experimental grounds as highly improbable.[111,113]

The favor in which axonal entry and viremic entry, respectively, have been held by students of poliomyelitis has alternated in remarkable fashion for nearly half a century. A cursory review of the literature shows that viremic invasion was advocated by Harbitz and Scheel[91] in 1907, formulated by Draper[32] in 1917, and recently revived by Bodian[11] in 1952. Nerve-borne invasion was suggested by Landsteiner and Levaditi[135] in 1909, upheld (as of major importance) by Flexner and Amoss[70] in 1914; defined as axonal by Fairbrother and Hurst[69] in 1929; formulated by Faber[37] in 1933, and supported by the experimental studies of Howe and Bodian[108] in 1941, who showed among other things that in the CNS the neurons constitute the only tissue susceptible to the virus The currently popular view that invasion of the CNS occurs by way of the blood is based on the fact that viremia appears to be at least fairly frequent during the early stages of infection.

In regard to the two divergent concepts, an illuminating statement by Flexner and Amoss[70] in 1914, based on their *experimental observations*, merits thoughtful consideration They said "it is established that experimental polio-

myelitis may be caused with more or less regularity by insuring that the virus reaches the central nervous system by way of the peripheral nerves When the virus is brought directly into relation with the central nervous system by intracerebral and intraspinous injections the most constant results are secured . . . When the virus is brought to the nervous organs by means of the blood, it is at first separated from the tissues themselves by the vessels and other structures interposed between the blood itself and the nervous tissue. For this reason it has been observed that, while small or even infinitesimal doses of the virus suffice to induce quite constant infection by the intranervous mode of inoculation, large quantities of the virus produce only occasional and inconstant infection, when injected directly into the blood The cause of this discrepancy has already been traced to an apparent inability of the virus to enter directly the substance of the brain and spinal cord from the blood" These authors estimated that the amount of virus required to produce CNS infection by the intravenous route was 1250 times greater than that by intraneural inoculation Flexner's view that the virus must first enter the cerebrospinal fluid and thence penetrate into the "interstices of the tissues" is no longer acceptable, since it has been shown by Howe and Peele[111] that when placed on the intact pia-arachnoid surfaces it does not cause infection

The barrier between the blood stream and the nerve cells, both in the CNS and in the peripheral ganglia has been discussed under Anatomical Considerations It appears to be a defense of some importance not only in poliomyelitis but in various other infections accompanied by viremia or bacteremia

The issue can best be resolved by comprehensive, day-by-day histological examinations of the CNS (brainstem and cord) after various kinds of peripheral exposure, in

search for the type and distribution of initial or very early lesions. This has been attempted by us with five different methods of exposure: 1) infraorbital nerve dip[63] (application of virus to the central end of the divided maxillary branch of the trigeminal nerve just external to the infraorbital foramen); 2) tonsillectomy[58] immediately following the application of virus to the tonsillo-pharyngeal surface; 3) gentle swabbing of the oropharynx with virus;[65] 4) oral administration of virus mixed with food,[66] and 5) intravenous and intra-arterial inoculation of virus.[47b] Cynomolgus monkeys (except in 1) and type 1 virus were used throughout. In 1) the relatively mild Cam strain was employed, and in the rest, Wis '45, a highly invasive and severe strain giving a high percentage of paralytic takes even by simple feeding The results follow

INFRAORBITAL NERVE DIP

In rhesus monkeys, the right maxillary nerve (infraorbital, afferent V), was divided and exposed for ten minutes to virus suspension, taking care to avoid contamination of the skin, and the wound was sponged and then carefully sutured

At two days, three of the four animals showed neuronophagia and focal infiltrations in the ganglia, but no lesions in the CNS. One animal escaped infection, showing no significant lesions anywhere Parallel subinoculations of the gasserian ganglia gave negative results throughout.

At three days, one animal showed no lesions of significance In three, marked typical lesions were found in the gasserian ganglia, more severe than those of the second day In parallel subinoculations of the gasserian ganglia, virus was detected in the ipsilateral but not in the contralateral side In one of the three animals with significant lesions in the ganglia, minute parenchymal and perivascular infiltra-

tions were found in the CNS which were limited to the spinal V nucleus in the pons No CNS lesions were found in the others

At four days, the gasserian ganglia showed typical lesions

TABLE XVI
INFRAORBITAL NERVE DIP EARLY LESIONS IN CNS

Day	*4*	*5*	*7*	*7*
CNS				
Diencephalon	0	0	0	0
Midbrain	0	0	v	0
Pons				
Reticular formation	0	0	+vv	v
Main sensory V	0	0	0/++vv	0
Spinal V	+/±	0/v	+v/++vv	v/vv
Vestibular nuclei	0	0	+/0	0
Motor V	0	0/v	0	0
Motor VII	0	0/+v*	v/++v**	0/+*
Other centers	0	0	0	0
Medulla				
Reticular formation	0	0	+vv	v
Spinal V	+/±	0	v/+v	0/v
Solitary (X)	0	0	vv/vv	0
Vestibular	0	0	0/+	0
Other centers	0	0	0	0
Spinal cord				
Cervical, thoracic, lumbar	0	0	0	0
Gasserian ganglia (V)	+++***/++**	+++***/+++***DD	+*/++**	++*/+++***

parenchymal infiltration v perivascular infiltration (cuffing) neuronophagia Left and right sides separated by /

in all four animals, but CNS lesions were found in only one These consisted again of parenchymal and perivascular infiltrations limited to the spinal nuclei of N V in both pons and medulla (Table XVI) No parallel subinocula-

tions of the gasserian ganglia were made at four days.

At five days, all four animals showed typical and severe lesions in the ganglia Parallel subinoculations of the gasserian ganglia were positive for both the ipsi- and the contralateral sides In one monkey, no lesions were found in the CNS. In one, which had begun to show tremors and right facial weakness, lesions were extensive and severe in the brain stem, mainly pons and medulla, but had not yet reached the cord. the trigeminal nuclei, reticular formation, the vestibular nuclei and the motor nuclei of cranial V, VI, VII, X (ambiguus) and XII were involved, the right facial most heavily In this case the exact situation of the initial foci in the CNS was obscured by secondary spread of infection, but was limited to the lower brainstem. In another animal, lesions were less far advanced, affecting the main and spinal trigeminal nuclei in the pons, the mesencephalic V nucleus and the locus coeruleus (also belonging to the trigeminal system) the motor V, VII and X nuclei also showed lesions In this case, there was a strong suggestion of entry through the trigeminal system In a third, with the exception of a perivascular infiltration in or near the motor V nucleus and of a small infiltrate in the spinal V nucleus, lesions were limited to the right facial nucleus, which were not severe, but included a little neuronophagia. Here, entry was almost certainly by the trigeminal route.

Of nine animals sacrificed *at six to ten days* after exposure, all showed typical lesions in the gasserian ganglia Five had developed paralytic symptoms (four with right facial paralysis) and all had advanced lesions throughout the brainstem, and some in the cord, limited in one case to the cervical level. While the trigeminal centers were involved in all five, the extent of the pathological process in the CNS made it impossible to discern the exact initial site of entry, but again this was probably in the brainstem.

Two animals showed no lesions in the CNS, despite the presence of lesions in the gasserian ganglia. In the remaining two, the process in the CNS was in its early stages, and apart from a few small perivascular infiltrations in the midbrain in one, was limited to the pons and medulla (Table XVI). In one the lesions were confined to the spinal V nuclei and reticular formation of the pons and medulla and the right facial nucleus, where slight degrees of parenchymal infiltration and neuronophagia were noted. In the other, the same centers were involved and also the solitary and vestibular nuclei to a slight degree, the right facial nucleus showed a more advanced and severer grade of involvement than in the preceding case. No lesions were found in the spinal cord in either of these two.

Summary of Nerve Dip Experiments

Centripetal axonal spread of infection following dire[ct] exposures of an afferent nerve was shown to occur wi[th] regularity as far as the peripheral ganglion, in which histo[logical] signs of infection were found as early as two days, and detectable virus as early as three days after exposure. However, further centripetal spread into the CNS, as determined by the presence of lesions, did not occur in all cases nor always on the same day. When it did occur, the earliest lesions were small and confined to the trigeminal centers. In one case, these were seen on the third, one on the fourth day, more often they were detectable on the fifth and subsequent days, although at this period infection had in several instances extended so greatly as to obscure the initial lesions. A notable and nearly constant feature of the early spread within the CNS was the involvement of the facial motor nuclei (cranial VII). In all instances the trigeminal nuclei were also involved, obviously by centripetal ascent through the afferent axons of cranial V, to

which the initial exposure had been confined. The early involvement of the motor nucleus of VII was presumably due to the fact that the trigeminal afferent and the facial efferent centers are intimately associated (see Fig 15) anatomically and functionally through collaterals and intermediate neurons which afford direct lines of communication for viral invasion. A few fibers of the facial (motor VII) nerve are said to join the infraorbital nerve at or near the foramen These may also have been infected during nerve-dip and led infection directly to the facial nucleus

This group of experiments elucidates the process of axonal invasion of the CNS from peripheral sources The grossly traumatic method employed has its counterpart in some cases of post-traumatic human poliomyelitis The localizations of the lesions in the CNS will be of interest by comparison with those observed in the following groups, exposed by other methods.

TONSILLECTOMY

The following experiments, which have not been previously published, were conducted with the purpose of determining the nerve paths through which virus entered the CNS after this operation As is well established in human beings and also in experimental animals tonsillectomy is frequently followed by bulbar paralysis, and we had surmised that the pathway of invasion might be the motor nerve supply of the paratonsillar musculature, leading to the nucleus ambiguus in the medulla, rather than the afferent fibers of the tonsillar area

Cynomolgus monkeys (M irus) and the Wis '15 (type I) strain of virus were used Under anesthesia, the tonsillar surface was swabbed with virus suspension and immediately thereafter the tonsils were removed Seventeen animals were thus treated, of which ten were sacrificed before

any clinical symptoms developed, and seven on the day of symptomatic onset. They were sacrificed under anesthesia by exsanguination, followed by perfusion with formalin in the usual manner.

Careful histological examination was made of the CNS by semiserial section and of the ganglia by complete serial section in 10 tonsillectomized monkeys without symptoms two at three days, three at five days, two at six days, and four at seven days after operation Similar examinations were also made of seven animals sacrificed on the day of onset of symptoms

Of the ten asymptomatic animals, only two showed significant lesions in the CNS, but of the eight without such lesions, six showed moderate lesions of significant character and degree in the peripheral ganglia, mainly in the gasserian, nodose and superior cervical sympathetic The relation between the central and the peripheral ganglionic lesions in the two asymptomatic cases in which both were present is shown in Table XVII In one (#721) entry had occurred *via* both the trigeminal and vagal afferent nerves, more heavily by the latter, and the only motor center affected was the ambiguus (X) in which, however, the lesions were slight The other (#723) is one of those rare but pathogenetically important instances in which invasion of the CNS by a single nervous pathway has been detected at its earliest moment, before secondary extension has occurred to mask the initial site of entry It demonstrates with precision the passage of invading virus from the nodose (vagus) ganglion into the connecting center in the medulla, the solitary nucleus, to which (with the exception of a single infiltrate in the reticular formation) the CNS lesions were confined

In the eight animals showing signs of paralysis and sacrificed at the onset the distribution of paralyses was mainly

TABLE XVII

LESIONS AFTER PHARYNGEAL SWABBING AND TONSILLECTOMY NO SIGNS OR SYMPTOMS OF POLIOMYELITIS TWO CYNOMOLGUS MONKEYS

Days After Operation	Monkey #721 3	#723 7
Pons		
Reticular formation	0	+
Locus coeruleus	0/+	0
Spinal V	vv/v	0
Motor centers	0	0
Medulla		
Reticular formation	0	±
Spinal V nucleus	vv/v	0
Solitary nucleus (IX, X)	+++vvv/ +++vvv	vvv/++
N ambiguus (motor X)	++/0	0
Other motor centers	0	0
Spinal cord	0	0
Ganglia		
V (gasserian)	++++***/	+/++*
VII (geniculate)	++*/+++**	0/0
IX (petrosal)	+++***/ +++***/	0/0
X (nodose)	++***/ +++***	+++**/+++*
Superior cervical sympathetic	+++*/ +++**	0/++
Celiac (intestinal sympathetic)	++*	++

v perivascular cuffing + parenchymal infiltrate * neuronophagia
0 no lesions Left and right sides separated by /

bulbar, as follows cranial V, 2; cranial VII, 7 (unilateral 6), cranial X, 5 (palatal weakness or dysphagia 5, laryngeal weakness, 3) XI (neck) 3, cranial XII tongue, 2, arms 2; no thoracic or lumbar paralyses were observed In all these there were extensive lesions in the brainstem, involving most of the motor centers (excepting the dorsal motor nucleus of the vagus which was usually spared), the reticular formation and most of the afferent centers, and it was

impossible to detect a single point of initial entry. Involvement of the area postrema was not observed. In the cord, lesions were present in the cervical and thoracic levels in all but in the lumbar level in only three and heavy in only one The distribution of lesions in these cases therefore points to initial entry into the brainstem with subsequent descent of infection into the cord.

Summary

The data presented, particularly those in two very early cases, indicate that when tonsillectomy is performed in the presence of virus on the pharyngeal surfaces, invasion of the CNS occurs by centripetal, axonal spread through the regional nerve supply (V and X). Contrary to our expectations the ascending infection, with one exception, did not follow the direct pathway through traumatized motor nerves but occurred mainly through the afferent supply via the peripheral ganglia That the latter were the site of primary infection is evident from the fact that significant lesions were regularly present in them regardless of the presence or absence of lesions in the CNS

SWABBING OF THE OROPHARYNX

The experimental procedure and its effects upon the peripheral ganglia have been described under Primary Invasion The quantity of virus to which the surface was actually exposed was not large Both lesions and virus were detected in the ganglia, the former as early as two and the latter as early as three days after exposure Of thirteen monkeys, allowed to survive, ten (77%) became paralyzed seven to sixteen days later (average 10 0 days) Typical lesions were found in the CNS, as well as in the ganglia, in all of the latter but were too far advanced to indicate the site of initial entry Two showed no symptoms and were sacri-

ficed at twenty-six and thirty-three days, respectively, lesions were present in the ganglia but none was found in the CNS.

Sixty-nine of the monkeys, of which twenty-six had had previous olfactory section, were sacrificed before any symptoms had appeared and at the following periods after swabbing. two days, 8; three days, 11; four days, 12; five days, 12, six days, 12, seven days, 8; eight days, 4; nine days, 2 In all of these a comprehensive histological examination was made of the CNS, in which the brainstem was studied by semiserial sections 50 μ thick at intervals of 1 25 mm, and by several similar sections from each of the three main divisions of the spinal cord.

No CNS lesions were found in fifty-six (81%) of the sixty nine Some lesions, all but two in the brainstem, were found in thirteen (19%) but these were merely small infiltrations and perivascular cuffs without any evidence of chromatolysis, neuron necrosis or neuronophagia They were scattered rather indiscriminately through all levels of the brainstem but with somewhat greater frequency in the pons and medulla; in two instances they were limited to the spinal V nuclei They averaged about 3 5 per animal. They were notably less common in new animals than in those that had been in the laboratory for two weeks or more The percentage of animals showing lesions increased roughly according to elapsed time after exposure, as follows: two to three days, 1/19 (5%), four to five days, 6/21 (25%); six to seven days, 3/20 (15%), eight to nine days, 3/6 (50%) These figures suggest a relationship of lesions to exposure, although we have found similar minute lesions in unexposed monkeys, as have Howe, Bodian and Morgan,[11] in unexposed chimpanzees

The escape of the CNS from axonal invasion in so large a proportion of the animals exposed by swabbing, when one considers that the peripheral ganglia were proved to

have been regularly infected, is an interesting and pathogenetically significant phenomenon, indicating, as it does, that neural infection often stops at the ganglion level. This is supported to some extent by the data compiled in Fig 8, showing the tendency of the virus to diminish in the ganglia after five days following pharyngeal swabbing The phenomenon would appear to throw light on the nature of many asymptomatic infections in poliomyelitis. It is to be noted that in the swabbing experiments the amounts of virus to which the animals were exposed were much less than those used in the simple feeding experiments discussed below

Summary

Of eighty-one cynomolgus monkeys exposed by gentle oropharyngeal swabbing, sixty-nine were sacrificed two to nine days later without having shown any signs of illness Ten others became paralyzed after an average incubation period of ten days, and three survivors failed to develop paralysis Of the sixty-nine asymptomatic animals, sacrificed early, all had lesions or virus in the peripheral ganglia, but fifty-six (81%) had no lesions in the CNS and of the remaining thirteen, eleven showed minor, scattered infiltrations possibly due to a defensive reaction at the blood-neuron barrier, and two showed small lesions limited to the spinal V nucleus indicative of axonal entry *via* the trigeminal route from the gasserian ganglia

Taking into account the regular presence in this series of proved infection in the peripheral ganglia and its frequent absence in the CNS in the same animals it appears that nerve borne invasion often stops at the ganglion level, thus offering an explanation for many cases of asymptomatic poliomyelitic infection on a purely neural but peripheral basis This may be related to the tendency of ganglion infection to subside after a few days

SIMPLE FEEDING

Reference has been made under Primary Invasion and Viremia to experiments in which lesions and virus found *in the regional ganglia supplying the mouth and throat* as early as two and three days, respectively, after simple feeding. In eleven of these animals, *comprehensive histological* examinations of the brainstem and cord were made in the usual manner. One animal (fifth day) showed no lesions in *the CNS*; three showed only a single cuff, and three, two to three cuffs plus slight parenchymal infiltrations of irregular distribution in *the* brainstem; no signs of neuronal damage were found in any of these. In view of the known viremia, such lesions as were found might perhaps be regarded as barrier reactions, like those found in *the* CNS after swabbing Lesions, affecting mainly the white matter, found in one animal were unlike those of poliomyelitis and the case was excluded from the series as unsuitable

In three animals, one sacrificed on the fifth and two on the sixth day, there were signs of early CNS invasion with *sharply localized lesions of strikingly similar distribution* (Table XVIII) The spinal V nuclei, reticular formation and facial VII nuclei were infiltrated with small cells and *there was neuronophagia, limited to one side,* in the facial nuclei In one case, the main sensory trigeminal nucleus *contained polymorphonuclears and* the mesencephalic V nucleus showed infiltration, while in another case the locus coeruleus was involved No other motor centers than VII, either in the brainstem or cord, showed lesions Apart from a few scattered small cuffs in the upper brainstem, no other *lesions were found in the CNS*

The pathological picture in these three cases, therefore, was one of early invasion localized to the trigeminal system and its immediate secondary connections, this strongly

TABLE XVIII
SIMPLE FEEDING EARLY LESIONS IN CNS

Day	*5*	*6*	*6*
Diencephalon	0/v	v/v	v/0
Midbrain	0	0	v/vv+(mes V)
Pons			
Reticular formation	v++	0/vv	vv+
Locus coeruleus	0	0	++/v+
Main sensory	0		0/+(pmn)
Spinal V	0/v	0/vv	0/v
Vestibular nuclei	0	0	0/v
Motor V	0	0	v/0
Motor VII	+v/++vvv**	+v/++vv**	vv/++vv*
Other centers	0	0	0
Medulla			
Reticular formation	0/vv	v/v	vv
Spinal V	0/vv	0	0/v
Solitary (X)	0/v	0	0
Other centers	0	0	0
Spinal cord			
Cervical, thoracic, lumbar	0	0	0
Ganglia (subinoculation)			
Gasserian (V)	+	+	+
Nodose (X)	+	+	+
Blood (subinoculation)	+	+	+

suggested axonal invasion from the gasserian ganglia, which were shown to contain virus as early as the third day after feeding. Thus, despite the demonstrated presence of vi remia, the lesions failed to show the random distribution to be expected of blood stream invasion but, on the contrary closely resembled in localization those observed after grossly traumatic exposures (nerve dip, tonsillectomy) in which invasion of the CNS almost certainly followed neural routes. Although the nodose ganglion had been shown to contain virus as early as the second day, there was no histo-

logical evidence of CNS invasion by the vagal route, again illustrating, as did the swabbing experiments, the not infrequent failure of ganglionic infection to ascend into the CNS

It is interesting to note that the proportion of animals with histological signs of CNS invasion was much higher after simple feeding, which is generally accepted as a "natural" mode of exposure, than after oropharyngeal swabbing, which has been considered by some writers as an unnatural one. There is a suggestion here that localized exposures in the oropharynx accompanied by slight friction, in which comparatively small amounts of virus are used, might actually give a closer approximation to natural conditions than does ingestion of massive doses

Summary

Histological examination of the CNS in three cynomolgus monkeys exposed by simple feeding of poliomyelitis virus and sacrificed before the onset of symptoms revealed early and sharply localized signs of invasion by the trigeminal route with secondary spread limited to the facial motor nucleus and a few other centers directly connecting with the trigeminal system.

The fact that localizations of lesions were those of nerve-borne and not of blood-borne invasion is particularly impressive because viremia had been repeatedly demonstrated in the animals of the series The absence of signs of CNS invasion in seven other animals of the same series is of interest with reference to the observations on intravascular inoculation that follow

INTRAVENOUS INOCULATION

Six thousand PD_{50} of the Wis '45 strain were inoculated intravenously[illegible] into each of four cynomolgus monkeys received on the previous day from the supplier Two of these

were sacrificed three days and two, five days after inoculation, and a systematic histological examination of the prefrontal cortex, brainstem and spinal cord was made, the total number of sections for each of these regions being thirty-two, 140 and 601 respectively, divided about equally between the four animals No definite lesions were found in any of these One very minute parenchymal infiltration of dubious significance was found in the thalamus of each of two animals, but nothing else The area postrema was normal

Summary

Intravenous inoculation of large amounts of potent virus failed to produce signs of invasion of the CNS in four test animals

INTRA-ARTERIAL INOCULATIONS

Viremic invasion of the CNS must occur through the arterial supply, which consists of the carotid artery and the vertebral artery, with some overlapping through the circle of Willis The former supplies the uppermost portion of the brainstem, the corpus striatum and the anterior portions of the cerebral hemispheres, the latter, the lower portions of the brainstem (midbrain, pons and medulla) and the entire spinal cord, which are the regions mainly affected by poliomyelitis, as well as the posterior portion of the cerebrum Direct inoculation of these two vessels would therefore correspond anatomically with the natural routes of vascular exposure of the CNS, although it would be much more intense than the natural one and than experimental intravenous inoculation, in both of which much of the circulating virus is probably removed from the blood, mainly by the LRE

Experiments were performed by the arterial route for four purposes to evaluate the efficacy of the virus removing

mechanisms in the peripheral circulation by comparison with intravenous inoculation; to evaluate the efficacy of the blood-neuron barrier within the CNS; to determine *whether viremic penetration and invasion of the CNS* occurs at a particular site or sites of high permeability; and to study the comparative susceptibilities of the neuronal tissues in the two vascular beds. In all the experiments, the highly invasive Wis '45 strain was used, with individual *inoculations of about 6000 PD_{50}.*

A. Inoculation of the Carotid Artery

Four cynomolgus monkeys were inoculated, of which one was sacrificed 3 days, one 4 days, and one, 8 days later; these *had shown no symptoms One developed bulbospinal paralysis on the 6th day and was then sacrificed* From the first three, a comprehensive histological examination of the CNS, including the prefrontal cortex, striatum, thalamus, midbrain, lower brainstem and spinal cord was made In *the 3 day animal, no lesions at all were found* In the 4 day animal, a few minor perivascular and parenchymal infiltrations, without signs of neuronal damage, were found in the striatum, thalamus and midbrain, but none below this level In the 8 day animal, the only lesions were sharply localized and obviously due to an intrathalamic inoculation performed 33 days before. In the paralyzed animal, perivascular and parenchymal infiltrations were so widespread through the lower neuraxis as to obscure the point of initial invasion The area postrema was uninvolved except in the last of the four, in which a few cuffs were observed near the inner side

B. Inoculation into the Vertebral Artery

Four animals were used Two were sacrificed before symptoms appeared, on the 3rd and 5th days respectively;

one was found dead on the 4th day, and one was sacrificed on the 8th day when paralysis appeared. Systematic histological examination was made of the midbrain, pons, medulla and cervical cord. The results were in sharp contrast to those of the carotid inoculations, and may be summed up as showing extensive infiltrative lesions in the brainstem which increased in intensity from above downward. In the uppermost portions (midbrain and upper pons), there was little evidence of neuronal damage; in the pons and medulla, more, and in the cervical cord, it was very severe and extensive, with chromatolysis, neuron necrosis and neuronophagia, most marked in the anterior horns. In the pons and medulla, the motor nuclei showed occasional small areas of neuronal destruction but much less than in the cord, the reticular formation and vestibular nuclei contained scattered lesions, mainly infiltrative, but the pontile nuclei and olives were largely exempt. No lesions were found within the area postrema; although occasional cuffs of exudate were noted near its inner border, there was no indication that infection had spread from it to adjoining nuclear centers. In the three and four day animals, many polymorphonuclear leucocytes were found in the infiltrates and neuronophagic lesions, indicating early involvement.

Summary

Inoculation of virus into the carotid artery produced only a few, early lesions of mild character in the corresponding area of the CNS. On the other hand, inoculation, into the vertebral artery with similar doses of virus, produced extensive lesions in the corresponding area, which were particularly severe in the spinal cord.

Certain important conclusions can be drawn from these experiments. 1. Since the amounts of virus were similar in

the vertebral artery and intravenous inoculations (see preceding section), the radical difference in effects on the CNS indicated that the LRE is highly efficacious in removing virus from the circulation, even in non-immune individuals. 2. The difference between the effects of carotid and vertebral artery inoculations was probably due to the known difference in susceptibility of nerve cells to poliomyelitis virus in the two arterial beds, since the carotid supplies neuronal areas known to be much less affected in human poliomyelitis than are those supplied by the vertebral A difference in relative efficacy of the blood-neuron barrier in the two areas seems improbable The protective role of the blood-neuron barrier against viral invasion therefore appears to be of less importance than has been supposed 3. No evidence was obtained that the area postrema or other similar vascular structure provides a site or mechanism of special permeability to the virus On the contrary, the evidence is strong that invasion may occur through the blood-neuron barrier at any point where susceptible nerve cells are present.

The relevance of the vascular inoculation experiments to human poliomyelitis must be considered in the light of the clinical syndrome Since blood-borne invasion appears to have infection of the cord as an immediate and predominant sequel, paralysis should be the first clinical manifestation of the disease This, however, is true of only a small minority of cases As a rule, when paralysis occurs at all, it follows three or more days of prodromals that are suggestive of lower brainstem involvement, the same symptoms are characteristic of both the non-paralytic cases and of the first phase of diphasic cases They fit better with axonal than with viremic initial entry (see section on Pathogenesis of Clinical Manifestations). Initiation of paralysis could be explained by either mechanism

CENTRIFUGAL SPREAD OF VIRUS IN PERIPHERAL NERVES

While the centripetal spread of poliomyelitis virus has been known for many years and evidence for centrifugal spread from the infected CNS into spinal and cranial ganglia has been adduced,[16] the possibility of further centrifugal propagation into peripheral nerves has been seriously questioned by Sabin and Ward[17] who, in 1941, stated "that the centrifugal spread of virus, which is so common in rabies, does not appear to occur in human poliomyelitis." Their conclusions were based on a study of seven human autopsies in which certain tissues and organs—nasal mucosa, salivary glands, sympathetic ganglia, adrenals and cervical and mesenteric lymph nodes—failed to show virus on subinoculation. They did not, however, test peripheral nerve itself. In 1940, Burnet and Jackson[41] obtained positive tests for virus in vagus, sympathetic and sciatic nerves of two cynomolgus monkeys during the stage of paralysis, and in 1950 we,[43] too, obtained positive results with brachial, sciatic and vagus nerves, also in paralyzed cynomolgus monkeys. We[44] made a more extensive study of the phenomenon in 1952-3.

Two methods were used, both with the Wis 45 strain. In one (sciatic nerve dip) the central end of the divided left sciatic nerve was exposed to virus and the animals (cynomolgus monkeys) were later sacrificed in groups as follows: one at four days before symptoms appeared, one at four to seven days when symptoms first appeared and one at four to nine days, one day after the onset of symptoms when paralysis was usually present. Each group was subdivided into two subgroups from each of which like tissues (cord, contralateral root, ganglia and sciatic nerves, leg muscle, and blood) were pooled and tested for virus by

subinoculation. The results are shown in the following table.

It will be noted that in group 4C, with the longest intervals (seven and nine days) between exposure and sacrifice, virus was still demonstrable in the contralateral nerve but no longer in the leg muscle and blood. The distal portion

TABLE XIX
CENTRIFUGAL SPREAD AFTER SCIATIC NERVE DIP

	Before Onset		*Day of Onset*		*Day After Onset*	
Experiment No	3-A	4-A	3-B	4-B	3-C	4-C
Animals per pool	4	4	4	2	4	2
Days after inoculation	4	4	4, 6, 7, 7	5, 6	4, 5, 5, 5	7, 9
Subinoculations						
Cord	+ +	—	—	—	—	—
Contralateral						
Nerve roots, $L_4 - S_1$	0 0	0 0	+ +	0 0	+ +	+ 0
Spinal ganglia	+ 0	+	+ +	+	+ +	+
Sciatic nerve						
Proximal	0 0	0 0	+ 0	0 0	0 0	0 0
Middle	0 0	0 0	+ +	0 0	+ +	+
Distal*	0 0	0 0	0 0	+ 0	+ 0	0 0
Leg muscle	0 0	+	0 0	+	+	0 0
Blood	+ 0	+ 0	+	+	+	0 0

+ positive result with paralysis 0 negative result — not tested
Each + and 0 sign indicates one subinoculated monkey
* Tibial and common peroneal nerves with some of their branches

of the contralateral sciatic contained demonstrable virus on the day of onset and the day after onset but not before onset of symptoms.

In the other series, the centrifugal movement of virus was investigated after intracerebral (thalamic) inoculation in three groups of monkeys (two each) sacrificed, respectively, on the day of onset of symptoms (five days), on the day after paralysis began (six days), and of complete pa-

ralysis (seven days) In this series a more comprehensive search for virus was made including ganglia, several peripheral nerves, muscles (including the heart), blood, nasopharyngeal washings and intestinal contents. The results are shown in Table XX

Particularly noteworthy and significant in these experiments are the time relationships between inoculation and positions of demonstrable virus in various portions of the peripheral nerves, in the upper and lower alimentary tract, and in the blood The findings reveal a progressive outward spread of virus of remarkable extent arising from primary foci of infection in the CNS and culminating in excretion and viremia Viremia was clearly a secondary result of primary neural infection

The demonstration of centrifugal spread in peripheral nerves brings poliomyelitis into pathogenetic relationship with rabies[209] and with the Borna disease (encephalomyelitis) of cattle,[154] both of which display the same phenomenon It may properly be regarded as one of the basic features of the disease process and as related to certain aspects of the clinical picture It may also explain in part the recoveries of virus from certain "extraneural" tissues

Summary

Experimental proof has been obtained of far reaching and perhaps almost universal centrifugal spread of poliomyelitis virus from the infected CNS into and througho the length of peripheral nerves This was demonstrated i the trigeminal, vagus, superior cervical sympathetic, bra chial and sciatic nerves The experiments further showed that viral excretion in the pharynx and intestine is closely related to centrifugal nerve-borne spread of infection Finally, they showed that viremia can result from primary neural infection without participation of the alimentary mucosa

The similarity of the centrifugal spread of infection in poliomyelitis to that obtaining in rabies and Borna disease is pointed out. It also provides a basis for certain important clinical aspects of poliomyelitis.

TABLE XX

CENTRIFUGAL SPREAD AFTER INTRATHALAMIC INOCULATION

	Day of Onset	*Day After Onset: Partial Paralysis*	*Day After Onset Complete Paralysis*
Experiment No.	5-A	5-B	5-C
Animals per pool	2	2	2
Day of onset	5, 5	5, 5	6, 6
Day of sacrifice	5, 5	6, 6	7, 7
Subinoculations			
Ganglia			
Gasserian	+	+	+
Nodose	+	+	+
Cervical sympathetic	+	+ 0	+ 0
Celiac	0 0	0 0	0 0
Nerves			
Trigeminal*	+	0 0	0 0
Cervical sympathetic	+	0 0	0 0
Vagus	+	0 0	+
Splanchnic	0 0	0 0	0 0
Brachial**			
Proximal	+ 0	+	0 0
Middle	0 0	0 0	+ 0
Distal	0 0	0 0	+
Sciatic			
Proximal	0 0	0 0	+
Middle	0 0	+ 0	+
Distal	0 0	0 0	+
Muscles			
Arm	0 0	0 0	0 0
Leg	0 0	0 0	0 0
Heart	+ 0	0 0	0 0
Blood	+	+	0 0
Intestinal contents	+	0 0	+
Nasopharyngeal washings	+	0 0	+

* Maxillary and mandibular divisions

** Mainly median, ulnar and radial nerves proximal segment, from axilla to middle of humerus, middle, same, to elbow, distal, same, to wrist

PATHOGENESIS OF THE CLINICAL MANIFESTATIONS

As shown in Table XXI, the first symptoms of poliomyelitis appear four to twenty-one days (median, 11.2 days) after initial non-traumatic exposure and therefore reflect a fairly advanced stage of the infectious process, although not yet as a rule that of localized weakness or paralysis. Following Draper's[32] formulation it is still cus-

TABLE XXI

Incubation Period. Onset of Symptoms After Single Non-traumatic Exposures*

Days After Exposure	*Number of Cases*
4	2
5	1
6	2
7	4
8	4
9	3
10	2
11	6
12	2
13	6
14	4
15	3
16	0
17	1
18	3
19	0
20	0
21	2
22–34	0
35**	1
Total	46

* Thirty-eight cases from Casey (24a), 8 cases of newborn poliomyelitis from various reports in the literature, assumed to have been acquired at birth from an infected mother. All cases were paralytic.

** This interval is so remote from the last previous positive as to be u[illegible]er suspicion as having been due to an unknown intermediate exposu[illegible]

tomary in many quarters to regard the initial symptoms and signs as representing infection of unspecified extraneural tissue ("systemic," "general" "non-specific" symptoms), in support of which the recent discovery of viremia has been adduced; and the subsequent appearance of

TABLE XXII

FIRST DAY SYMPTOMS, EXCLUSIVE OF FEVER, IN ORDER OF FREQUENCY. 202 CASES

		*No.**	*%**	*Nervous Character*
I	Pain	145	73	+
	Headache	89	44	+
	Other localized pain**	116	47	+
	Generalized aching	5	2	±
II	Vomiting/nausea/anorexia	83	41	+
	Vomiting/nausea	56	28	+
	Anorexia	27	13	±
III	Malaise	32	16	±
IV	Stiffness of neck or back	31	15	+
V	Listlessness, fatigue, drowsiness	31	15	+
VI	Localized weakness or paralysis	20	10	+
VII	Chills	16	8	±
VIII	Fretfulness, irritability	13	6	±
IX	Vertigo	9	4	+
X	"Cold" (unspecified 1, nasal discharge 4)	5	2	0
XI	Diarrhea, dysuria, each	2	1	0
XII	Sweating, poor vision, constipation, each	1		±
	Cerebrospinal fluid positive in 5 of 10 cases Examined on 1st day			

* Number designates the number of times the given symptom or category was recorded, percentage, the proportion of total cases in which it occurred (avoiding duplication when more than one symptom of a given category was recorded in an individual case)

** Eyes (photophobia) 8, ears 2, throat 18, neck 20; upper extremities 5, back 22, chest 6, abdomen 16, lower extremities 19

"meningeal" signs (stiffness of neck, back and hamstrings), definite bulbar signs, and weakness of paralysis as marking the initial invasion of the CNS. The two-stage, or diphasic course, seen in 10-30% of cases, in which the symptoms temporarily subside and after a variable interval reappear,

usually in severer form, is also adduced in favor of this concept; the first phase ("minor illness") being held to represent the extraneural stage and the second, the stage of CNS invasion. Such an interpretation of the symptomatology could be valid only if it conforms to the character of the symptoms themselves, many of which, it may be suggested have been too casually accepted as "non-specific" or "general" in type. Are they, in fact, actually non-specific—that

TABLE XXIII

COMPARISON OF COMMONEST ONSET SYMPTOMS IN PARALYTIC, NON-PARALYTIC AND FIRST PHASE OF DIPHASIC CASES IN 202 CONSECUTIVE CASES OF POLIOMYELITIS

	Paralytic (120 cases)	*Non-paralytic (82 cases)*	*First Phase of Diphasic Form (20 cases)*
Headache	43%	47%	25%
Other localized pain	43%	46%	45%
Vomiting/nausea	30%	28%	30%
Anorexia	11%	8%	35%
Malaise	17%	14%	25%
Stiff neck or back	11%	15%	0
Listlessness/fatigue/lethargy/drowsiness	14%	16%	15%
Localized weakness or paralysis	10%	0	0
Irritability/restlessness	6%	5%	5%

is, identical with those of other acute infectious diseases of non neural character—or, on critical scrutiny are they found from the beginning to reflect a disturbance of the nervous system itself?

Horstmann[89] in 1949 published an account of the early symptomatology of 388 personally observed cases from New England and in 1950 I[90] analyzed a series of 202 cases from Northern California all personally observed by Dr Henry D Brainerd and placed by him at my disposal The

two series are in general agreement, but mine, because the first-day symptoms were separately listed, is perhaps somewhat more suitable for present purposes In Table XXII all the onset symptoms observed in the 202 cases are tabulated and in Table XXIII those of the non-paralytic and diphasic cases are presented separately in parallel columns

It will be seen that, with few exceptions, the onset symptoms are either neural in character or readily explainable on the basis of neural infection, and are closely similar in the paralytic and non-paralytic cases and in the first phase of the diaphasic cases Particularly notable in this, as in Horstmann's series, is the fact that *localized pain is the most frequent single symptom category at the onset*, occurring in our own series in nearly three-fourths of all cases Next in order were nausea, vomiting and anorexia, which were present in about 40% of all cases. Malaise, stiffness of neck and back, listlessness, drowsiness, weakness or paralysis, chills, fretfulness, irritability and dizziness (in descending order of frequency) accounted for nearly all of the remaining symptoms. Weakness or paralysis was already present on the first day in 10% of cases, sometimes following a few hours of premonitory symptoms. Signs of respiratory infection or of diarrhea were observed in only 2% and 0.5% respectively, of all the cases With these two infrequent exceptions, the constellation of initial signs and symptoms at the onset is referable to infection of the nervous system The identity of the clinical picture in the first phase of the diphasic cases with that of the onset of other cases, non-paralytic as well as paralytic, without an asymptomatic interval indicates that *the diphasic ("dromedary") phenomenon merely represents remission and relapse of the same basically neural process* In half of our cases the cerebrospinal fluid when tested on the first day of symp-

toms showed pleocytosis and increased protein—positive proof of CNS involvement. Negative findings in the fluid do not, of course, rule this out; in the same series the CSF remained normal throughout the course in 10% of the cases, including several with severe paralysis. In Bahlke and Perkins's study of gamma globulin therapy, 111 patients, examined during the early, non-paralytic stage, showed pleocytosis; of these 55% failed to develop paralysis, regardless of whether or not they received gamma globulin. Andelman, Fishbein and Casey,[2] and Casey, Fishbein and Abrams[24] showed that a number of patients with previous mild, non-paralytic attacks (minor illness) had pleocytosis or increased protein or both in the CSF.

When patients with supposedly non-paralytic poliomyelitis are examined and followed by comprehensive muscle surveys, a large proportion of them are found to have some evidence of paralysis. Moskowitz and Kaplan[151] found that in 39% of seventy-five "non-paralytic" patients examined one and one-half to six years later minimal weakness was present in one or more muscle groups, while Shaw and Levin,[155] by consecutive muscle surveys of patients over long periods following the initial attack found that nearly all the "non-paralytic" cases showed the same phenomenon.

Experimental observations on infected monkeys[174] and chimpanzees show that invasion of the CNS can and often does occur in the absence of detectible symptoms or physical signs, both in cases observed for long periods after exposure and in others in which, had the animals not been sacrificed, paralysis would almost certainly have supervened a day or two later. Further, animals beginning to show premonitory signs and symptoms, such as excitability and tremor, but without detectable weakness, almost invariably display characteristic lesions in the CNS, sometimes of striking severity and extent.[14]

These various data demonstrate that in human beings and in animals infected with poliomyelitis, involvement of the CNS not only often precedes paralysis but can occur independently of it, sometimes in patients with only minor symptoms not definitely pathognomonic of CNS disturbance. It seems highly probable that in human beings, as is demonstrably true in animals, *infection has entered the CNS in advance of and independent of the emergence of paralytic clinical manifestations and that the latter appear only after the pathological process has reached a critical level of severity Moreover, experimental evidence indicates that invasion of the CNS of limited extent may sometimes be present even in cases without any observed clinical manifestations.*

It is noteworthy that clinical manifestations (as well as pathological changes) referable to the tissues found most suitable for tissue culture are conspicuously absent in human poliomyelitis: dermatitis, nephritis, orchitis, and enteritis are either unknown or occur so rarely as not to constitute a significant part of the disease picture. Sore throat, found in about 10% of cases, is not accompanied by pharyngeal exudate or swelling and is probably of neurogenic origin The fact that enteritis does not form part of the clinical (or pathological) syndrome is of special interest in reference to the view that the alimentary mucosa is the site of primary and continuing multiplication of the virus

A tentative list of neural sites or origin of common clinical manifestations at the onset is given in Table XXIV. Many of these (headache, vomiting, nausea and anorexia; drowsiness and lethargy, stiffness of neck and back; affective disorders, and dizziness) are referable to the brainstem and constitute a syndrome of *non-paralytic brainstem ("bulbar") encephalitis* presumably correlated with the

TABLE XXIV

SUGGESTED ANATOMICAL SITES OF ORIGIN OF COMMONEST ONSET SYMPTOMS

Symptoms	*Origin*
Headache	Cranial ganglia, vessels of brainstem (pulsatory headache), meninges of brainstem (?)
Pain in neck, back, thorax, abdomen, extremities	Spinal ganglia, posterior roots, peripheral nerves, spinal cord vessels, meninges (?)
Vomiting/nausea/anorexia	Medulla (solitary nucleus, dorsal vagal nucleus ("vomiting center"), vestibular centers, hypothalamus
Stiffness of neck, back and hamstrings	Brainstem (reticular formation), internuncial centers in cord, spinal ganglia (with pain)
Sore throat	Gasserian ganglia, nodose ganglia
Drowsiness/lethargy	Hypothalamus thalamus
Affective disturbances anxiety, apprehension, malaise, emotional disorders	Thalamus
Dizziness	Vestibular centers in medulla and cerebellar roof nuclei
Abnormalities of pulse, respiration and blood pressure	Medulla (reticular formation)

characteristic distribution of lesions in this region Others (sore throat, pain in the neck, back, extremities) may be referable to the sensory ganglia, roots and nerves In only about 10% of cases are weakness and paralysis present at the onset to indicate advanced involvement of the motoneurons of the brainstem or cord The predominance of brainstem manifestations at the onset suggests the possibility that the initial invasion of the CNS occurs in this region, rather than in the cord, which would agree with

filtration and phagocytosis of the virus by reticuloendothelial tissues of the liver, lungs, spleen, bone marrow and lymph nodes, which remove virus from the circulation and lead to the manufacture of antibody; 2) the choroid plexuses, which block the entry of virus into the CSF, and 3) the blood-neuron barrier in the CNS, and also in the peripheral ganglia, which prevents circulating virus from invading nerve cells 1) and 3) have definite limitations in efficiency.

The concept of pathogenesis outlined in this paper has an important bearing on the scope and limitations of antipoliomyelitic immunization procedures.

Immunity to viral infections in general falls into two categories. humoral and cellular ("tissue") The protective value of humoral immunity is limited to extracellular phases of the infection, where it can neutralize (without necessarily immediately destroying) the virus before it enters axons and neurons and becomes inaccessible to antibody The chief extracellular situations in poliomyelitis are the alimentary lumen (and excreta) and the bloodstream. Bell[4] has shown that in immune individuals neutralizing antibody is detectable in the pharyngeal secretions almost constantly when it is present in the serum above the level of $10^{-1.8}$ (1/64) dilution of the 50% neutralization end point, at lower levels, it was still detectable in about one-third of cases. The mean ratio of antibody in the secretions to that in the serum was 1 25, with a median of 1·15. In another study Bell[9] demonstrated a straight-line logarithmic relationship between the quantity of virus and the quantity of immune serum required to neutralize it; thus, for small amounts of virus correspondingly small amounts of antibody were effective In poliomyelitis, as in other infectious diseases, the number of infective particles entering the body initially is in all probability extremely small

From these considerations it seems highly probable that artificially induced immunity could produce concentrations of specific immune substances in the overlying mucus that could oppose a barrier to initial invasion of the surfaces of the mouth and throat under natural conditions of non traumatic exposure; in other words, prevent primary invasion at the portal of entry. Both active and passive immunization can be expected to act in this way, the former more effectively and durably. However, no quantitative measurements of antibody after gamma globulin treatment in human beings have been reported, either in the blood or in the secretions. The distinctly limited prophylactic effects observed in field trials[83, 84] indicate that the dosage employed (0 3 ml./kg body weight) was near the lower effective limit.

There is no evidence that neutralizing antibodies are excreted in the intestine to perform a similar service there, but it is encouraging that our studies[51] indicated that paralytic poliomyelitis rarely follows exposures confined to the gastrointestinal mucosa.

It is theoretically possible, though unproved, that humoral antibodies might enter infected and damaged nerve cells and, perhaps even more remotely possible that they could then prevent further migration of the virus There is no evidence that circulating antibodies might block cell to-cell transfer of virus at the synapses, as I once suggested

The presence of neutralizing antibodies in the blood can be confidently expected to lessen or, if in adequate concentrations, completely to prevent blood-borne dissemination of virus[55] This action is of the greatest importance in limiting neuronal invasion from the blood of both the peripheral and central components of the nervous system

Immunization by vaccine composed of inactivated virus and depending solely on the production of humoral anti-

body thus appears to offer valuable but not complete protection against poliomyelitic infections*. The levels of antibody observed by Salk[179,180] after vaccinating individuals without pre-vaccination antibody (primary immunization) were low, but recall injections produced high levels. Inactivated vaccine has certain disadvantages which Sabin[171] has pointed out, of which its somewhat uncertain and probably limited duration is perhaps the most important. Experience with other inactivated viral vaccines, notably influenza,[96] indicates that the protection provided by them lasts only a few months and is in contrast with the more enduring effect produced by vaccines (vaccinia, yellow fever) containing living, attenuated virus. With inactivated poliomyelitis vaccine, recall injections will undoubtedly be necessary at intervals not yet determined but probably of the order of a year or less. Moreover, in view of the apparently greater risk of paralytic infections in adults, it may be necessary to continue recall injections throughout life.

An important question, as yet unanswered, is whether individuals immunized with inactivated vaccine might again become susceptible later on when antibody titers had declined below critical levels, and whether they would on subsequent exposure be able to respond with a prompt rise in antibody adequate to keep a second infection at the asymptomatic level. Theoretically, such a booster effect could not occur quickly enough to prevent oropharyngeal neural entry by antibody blockade in the over-

* The report by Francis of the 1954 field trials of the Salk vaccine appeared as this volume goes to press. Against paralytic poliomyelitis protection was about 60-70% for infections with Type 1 virus and over 90% with Types 2 and 3. Non-paralytic infections were less affected. The results suggest good protection against viremic invasion of the CNS and poor protection against primary neural implantation. Probably only primary immunization, with low antibody levels, was induced in many cases

lying mucus, and therefore the possibility of nerve-borne transmission of infection to the peripheral ganglia and thence to the CNS would remain open. It might, however, occur in time to lessen, if not completely remove, the hazards of viremia. Obviously, the question cannot be answered by human evidence for some years. The subject deserves further investigation.

Enduring immunity to viral infections in general and to poliomyelitis in particular depends on actual infection, whether clinically inapparent or manifest. Asymptomatic infection with solid immunity can be reproduced in animals by simple exposure of the alimentary mucosae to virus. In 1913 we[19] subjected a series of five cynomolgus monkeys, over periods of nine to fourteen months, to repeated exposures of the alimentary surfaces (by oronasal spray, feeding, enema) with the Per strain (Type 1). None of these having shown signs of infection, all were challenged a month after the last exposure by intracerebral inoculation with the same strain, the severest possible test. Of the five only one (20%) developed paralysis, in contrast to 92% of twenty five previously unexposed monkeys also inoculated intracerebrally with Per. The character of the induced resistance in the four refractory animals was revealed by histological examination of the CNS. It consisted, not of complete absence of inflammatory reaction but rather of a strikingly limited spread of infection from the site of inoculation, with descent in moderate degree to the brainstem and very little (two cases) or none (two cases) to the cord. The severity of the test by intracerebral challenge as well as the character of the reaction to it indicate that in these experiments immunity was cellular rather than humoral. Subsequently, Melnick and Horstmann,[120] and Howe, Bodian and Morgan[119] induced immunity in chim

panzees by simple virus feeding with the production of active infection, mostly asymptomatic. However, paralytic infection did occur in some instances.

The search for safe attenuated immunogenic strains which has gone on since 1910 did not succeed until 1951 when Koprowski and his associates[129] discovered a strain (TN) of Type 2 virus without paralyzing effects but with immunizing properties when fed This strain has now been administered orally to a considerable number of human subjects with encouraging results.[129,130] Other investigations[35,171] are in progress using tissue culture in the search for immunogenic mutants of all three types with low paralyzing properties, also with encouraging results Tissue immunity, it may be added, is the only condition that offers reliable protection against axonal conduction of infection.

RECAPITULATION

DATA presented in the foregoing sections support each of the following conclusions regarding the pathogenesis of poliomyelitis

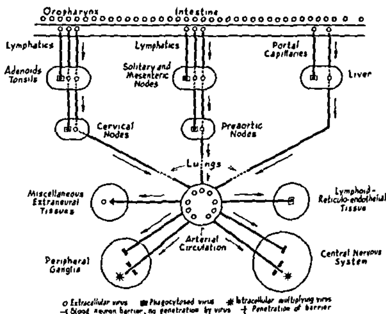
1 Poliomyelitis is not an airborne disease and the virus ordinarily enters the body through the oral rather than the nasal passages In exceptional instances it is introduced through the skin or mucous membrane by trauma

2. The virus is basically neuronocytotropic and its primary host in the living subject is the nerve cell alone Only drastic chemical or hormonal modifications of the extracellular environment permit it to infect and produce pathological changes in other tissues In human disease, such modifications are very unlikely to occur excepting after neuronal involvement is advanced and severe Extraneural infection in poliomyelitis, if or when it occurs, must therefore be a late, secondary phenomenon, not a primary or early one

3 The initial invasion of the body tissues ordinarily occurs into the peripheral nerves of the mouth and pharynx, perhaps aided by friction (hard foods, tooth brush), followed by centripetal spread to the regional peripheral ganglia, particularly the gasserian (afferent trigeminal), nodose (afferent vagal), and superior cervical sympathetic

4 The primary site of viral multiplication and lesions is the peripheral ganglia In many, but not all, asymptomatic cases infection may extend no farther than this

5 Excretion of virus into the alimentary lumen (mainly the oropharynx) is secondary to infection of peripheral ganglia, whence the virus is conveyed by centrifugal axonal spread to the surface From the oropharynx it reaches the lower alimentary tract by swallowing In later stages it may also be excreted directly into the intestine from the celiac



STAGE 3 PASSIVE ABSORPTION AND VIREMIA

FIGURE 13 Virus excreted into the alimentary lumen in the extracellular state is passively absorbed by two routes: 1) into the lymphatics of the oropharynx and intestine, and 2) into the portal capillaries of the intestine Part of the virus passing through the lymphatics is taken up by the reticuloendothelial tissue in the lymph nodes, and phagocytosed (or stored), thus inducing formation of antibodies and part of it reaches the systemic circulation *via* the venae cavae and lungs, producing viremia Part of the virus entering the intestinal capillaries is sequestered by the reticuloendothelial cells of the liver, and part of it reaches the systemic circulation, *via* the inferior vena cava and lungs Again the reticuloendothelial tissues (spleen, lymph nodes, etc) remove part of the virus from the circulation, to be phagocytosed with antibody formation, or passively stored Another part reaches the nervous tissues (CNS, peripheral ganglia) here the blood neuron barrier (microglia, astrocytes, oligodendrocyte capsule cells) partly prevents viral contact with neurocytes while penetration of the barrier leads to their invasion and infection During the viremic stage, virus might theoretically be detectable in any of the extraneural tissues, depending on its concentration in the blood and the amount of blood in the test sample

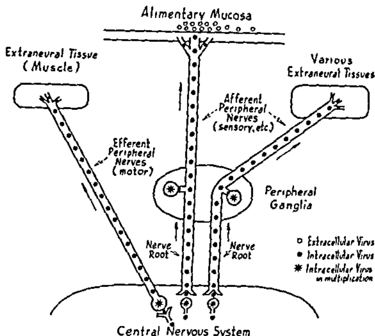
plexus, and possibly also from the submucous plexus, although the latter has not been proved. Excretion is not due to multiplication in the non-neural elements of the alimentary mucosa.

Excretion is a continuing, cyclic process, of increasing magnitude during the early stages of the disease and results (a) in reinvasion of the peripheral nerves and ganglia, and (b) in viremia In the later active, and convalescent stages, it persists for a considerable period, possibly as a result of continuing centrifugal nerve-borne migration

6 Viremia of detectable proportions occurs when excretion of virus has attained a considerable magnitude and amounts of virus are passively absorbed through the intestinal walls sufficient to exceed the phagocytic capacities of the LRE (liver, lungs, spleen, lymphnodes, etc). It becomes important in spreading infection within the body when virus reaches the arterial circulation Viremia ends when specific immune antibodies are formed and attain adequate concentrations in the blood

7 Invasion of the CNS occurs (a) when infection of the peripheral ganglia is severe enough and involves enough neurons to favor the ascent of infection through their central axons into the corresponding afferent centers, mainly in the brainstem, and their immediate connections, (b) when considerable amounts of virus are traumatically introduced into peripheral nerves (tonsillectomy, injections, etc), and (c) when viremia is intense enough to break through the blood-neuron barrier in the CNS There is no evidence that the area postrema is a site in which the circulating virus enters the parenchyma of the CNS

8. After infection has become established in the CNS the virus spreads centrifugally into peripheral nerves generally, reaching their distal segments and then contributing to the continuance of excretion



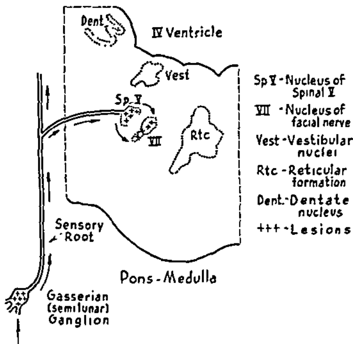
STAGE 4 CENTRIFUGAL AXONAL SPREAD FROM THE CENTRAL NERVOUS SYSTEM

FIGURE 14 As infection becomes established and disseminated in the CNS, virus spreads centrifugally and extensively through the axons of peripheral nerves, reaching their most distal portions In afferent nerves, this occurs by way of the peripheral ganglia, while in efferent nerves virus passes directly from the CNS to the muscles, skeletal, cardiac and unstriated Secondary excretion accompanies this process by way of afferent nerves Some virus also escapes from the CNS into the venous blood

By this process of neural dissemination, as well as *via* the blood, virus can reach most of the extraneural tissues and be detected therein

Centrifugal spread in nerves may supply a clue to the long continued excretion of virus

9 The tenure of the intracellular, vegetative phase of poliomyelitic viral activity is commonly brief and tenuous, hence the progress of active infection may cease at any



AXONAL INVASION OF THE CENTRAL NERVOUS SYSTEM

FIGURE 15 This semidiagrammatic figure illustrates axonal ascent of infection by the trigeminal (V) route from the gasserian ganglion to the spinal V nucleus in the lower brainstem, with secondary spread to the facial nucleus The adjacent vestibular and cerebellar roof nuclei and reticular formation are frequently involved at an early period, also by secondary spread Centripetal invasion by the vagal route from the nodose ganglion to the solitary nucleus in the medulla and thence to the nucleus ambiguus, reticular formation, etc is comparable See Tables XVI, XVII and XVIII

point This apparently most often occurs after initial infection of the peripheral ganglia, resulting in subclinical infection It may occur shortly after entry into the CNS, resulting in abortive and other non-paralytic infections

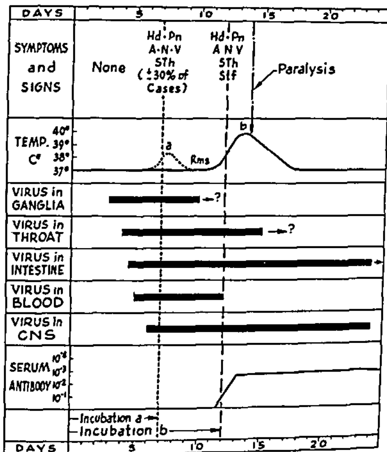


FIGURE 16 The diagram is partly based on those of Horstmann[m] and of Bodian[ib] for purposes of comparison, but with important differences based on our own experiments and clinical observations, of which the following are particularly to be noted A The first hump of the diphasic cases is represented as an exceptional, not

with mild symptoms ("minor illness"), and it may occur after more advanced invasions of the CNS which end in various degrees of recovery from paralysis. It is improbable that overt clinical manifestations of poliomyelitis occur without involvement of the CNS Nearly all the symptoms at the onset and later are either characteristically neural or readily explainable on the basis of neural infection

10. Immunization induced by vaccine can prevent or mitigate poliomyelitis in two ways 1) by neutralizing the virus and thus blocking initial entry in the oropharynx through the agency of antibodies excreted in the overlying mucus, and 2) by neutralizing virus in the blood stream after initial infection has occurred Passive immunization by gamma globulin can act in the same ways, provided the dosage is large enough, but only for a short period of time

Various phases and aspects, consecutive and concurrent, of poliomyelitic infection are illustrated in Figures 11 16

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a constant, feature of the disease, and as occurring *after* invasion of the CNS has begun Note the similarity of the symptoms in the two phases B The order of appearance of virus in the various locations is different, as follows 1) peripheral ganglia 2) throat, 3) intestine, 4) blood, 5) CNS Compare this order with Bodian's [115] 1) intestine 2) throat, 3) blood, 4) CNS The diagram is not intended to indicate that CNS invasion occurs only by the vascular route, but that it would occur at about the same time either by that or by the neural route

The term "incubation period' is defined as the interval between initial exposure and the emergence of the first symptoms It varies considerably from case to case For convenient representation two different periods, a and b are shown in the diagram to indicate the time relationship between the first and second humps" in the diphasic cases, and the average time of onset of symptoms in the commoner monophasic cases, however, the incubation periods in the two types are not necessarily different from each other

BIBLIOGRAPHY

1 Abramson, H, Greenberg, M, and Magee, M C · Poliomyelitis in the newborn infant *J Pediat*, *43*,167, 1953
2 Andelman, M B, Fishbein, W. I, and Casey, A E: Spinal fluid in the retrospective diagnosis of subclinical poliomyelitis *South M J*, *39* 706, 1946
3 Aycock, W L, and Amoss, H L· Experiments on local specific therapy in poliomyelitis, the utilization of hypertonic solutions in the serum treatment of experimental poliomyelitis Infantile Paralysis in Vermont, 1894-1922, A Memorial to Charles S Caverly Burlington, Vermont, State Dept of Public Health, 1924
4 Bachrach, H L, and Schwerdt, C E Purification studies in Lansing poliomyelitis virus II Analytical electron microscopic identification of the infectious particle in preparations of high specific infectivity *J Immunol*, *72* 30, 1954
5 Bahlke, A M, and Perkins, J E Treatment of preparalytic poliomyelitis with gamma globulin *J.A.M.A.*, *129* 1146, 1945
6 Baker, A B, Brown, I A, and Cornwell, S· Poliomyelitis VII Gastrointestinal disturbances their relationship to lesions in the hypothalamus *J Nerv & Ment Dis*, *116* 715, 1952
7 Baskin, J L, Soule, E H, and Mills, S D: Poliomyelitis of the newborn Pathologic changes in two cases, *Am J Dis Child*, *80* 10, 1950
8 Bell E, J The relationship between the antipoliomyelitis properties of human nasopharyngeal secretions and blood serums *Am J Hyg*, *47* 351, 1948
9 ——— Experimental studies of variables in neutralization tests with Lansing poliomyelitis virus *Am J Hyg*, *48* 381, 1948.
10 Bloomfield, A I The dissemination of bacteria in the upper air passages I The circulation of foreign particles in the mouth *Am Rev Tuberc*, 5 903 1921-22
11 Bodian, D (a) A reconsideration of the pathogenesis of poliomyelitis. *Am J Hyg*, *55*,414 1952, (b) Pathogenesis of poliomyelitis *Am J Pub Health* *42* 1388, 1952
12 ——— Histopathologic basis of clinical findings in poliomyelitis. *Am J Med*, *6* 563, 1949
13 Bodian D, and Cumberland, M C The rise and decline of poliomyelitis virus levels in infected nervous tissue *Am J Hyg*, *45* 226 1947
14 Bodian D and Howe H A The pathology of early arrested and non paralytic poliomyelitis *Bull Johns Hopkins Hosp*, 69 135, 1941

15 ——. The rate of progression of poliomyelitis virus in nerves *Bull Johns Hopkins Hosp*, 69 79, 1941

16 ——. The significance of lesions in the peripheral ganglia in the chimpanzee and in human poliomyelitis *J Exper Med*, 85 231 1947

17 Bodian, D, and Paffenbarger, R S Viremia and antibody response of abortive human cases *Federation Proc*, 12 437, 1953

18 Bodian, D Experimental studies on passive immunization against poliomyelitis III Passive active immunization and pathogenesis after virus feeding in chimpanzees *Am J Hyg*, 58 81, 1953

19 Bower, A G, Morgan, F M, and Chaney, A L Nitrogen and potassium metabolism in poliomyelitis *Am J Med Sc*, 223 532, 1952

20 Brodie, M Fall of infectivity and immunizing power of poliomyelitic tissue at intervals after complete paralysis *Proc Soc Exper Biol & Med*, 30 1238, 1933

21 Burnet F M, and Jackson, A V Poliomyelitis, spread of poliomyelitis virus in cynomolgus monkeys with particular reference to infection by the pharyngo intestinal route Austral *J Exper Med & Med Sc* 18 361, 1940

22 Burrows M T Is poliomyelitis a disease of the lymphatic system? *Arch Int Med*, 48 33, 1931

23 Cammermeyer J Is the human area postrema a neuro vegetative nucleus? Acta anat 2 294, 1946 47

24 Casey A E, Fishbein W I, and Abrams, I Relative frequency of "subclinical poliomyelitis *Am J Dis Child*, 72 661 1946

24a Casey, A E The incubation period in epidemic poliomyelitis *J.A.M A*, 120 805, 1942

25 Charcot J M, and Joffroy, A Cas de paralysie infantile spinale avec lésions des cornes antérieures de la substance grise de la moelle épinière *Arch physiol norm et path*, 3 134, 1870

26 Clark, P F, Fraser, F R, and Amoss, H L The relation to the blood of the virus of epidemic poliomyelitis *J Exper Med* 19 223 1914

27 Clawson, B J, Noble, J F and Lufkin N H Nodular inflammatory and degenerative lesions of muscles from 450 autopsies *Arch Path*, 43 579, 1947

28 Committee on Nomenclature of the National Foundation for Infantile Paralysis A proposed provisional definition of poliomyelitis virus *Science*, 108 710 1948

29 Darrow, D C, and Miller H D The production of cardiac lesions by repeated injections of desoxycorticosterone acetate *J Clin Investigation*, 21 601 1942

30 Delbruck M Cold Springs Harbor symposium on the biological nature of viruses *Science*, 118 66, 1953 reported by Mark H Adams

31. Denst, J. and Neubuerger, K T.: A histologic study of muscles and nerves in poliomyelitis *Am. J Path*, *26 863*, *1950*
32 Draper, George. *Acute Poliomyelitis* Philadelphia, Blakiston, 1917
33 Enders, J F · In T M. Rivers (Ed): *Viral and Rickettsial Infections of Man.* Philadelphia, Lippincott, 1948, p 121
34 Enders, J F, Weller, T. H, and Robbins, F C · Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. *Science*, *109* 85, 1949.
35. ——— Alteration in pathogenicity for monkeys of Brunhilde strain of poliomyelitis virus following cultivation in human tissues *Federation Proc*, *11* 467, 1952
36 Evans, C A, Byatt, P H, Chambers, V C, and Smith, W. M: Growth of neurotropic viruses in extraneural tissues. VI Absence of in vivo multiplication of poliomyelitis virus, types I and II, after intratesticular inoculation of monkeys and other laboratory animals *J. Immunol*, *72* 348, 1954
37 Faber, H K Acute poliomyelitis as a primary disease of the central nervous system *Medicine*, *12* 83, 1933
38 ——— Visualization of paralytic lesions of poliomyelitis by intravital staining *Proc Soc Exper Biol & Med*, *35* 10, 1936
39 ——— Adenotonsillectomy and poliomyelitis Editorial, *Pediatrics*, *3*. 255, 1949
40 ——— Pathogenesis and onset symptoms of poliomyelitis *Pediatrics*, *6* 188, 1950.
41 ———. Postinoculation poliomyelitis Editorial, *Pediatrics*, *7* 300, 1951.
42 ———: The question of extraneural growth in vivo of poliomyelitis virus *Science*, *114* 570, 1951
43 ——— Neonatal poliomyelitis after known exposure; *15 cases from the literature* Unpublished data, 1953.
44 ——— Personal observations on terminal nerve filaments in alimentary mucous membranes *Unpublished data*
45 Faber, H K, and Dong, L Inactivation of poliomyelitis virus in relation to gastric and intestinal digestion *Proc Soc Exper Biol & Med*, *63* 575, 1946
46 ———. Personal observations on viral content of mesenteric nodes after feeding virus Unpublished data. 1955
47 ——— Origin and effects of viremia in poliomyelitis. In preparation
47a ——— Studies on entry and egress of poliomyelitic infection VII Early lesions in peripheral ganglia after simple feeding with comments on the possible value of immunization in preventing neural entry *J Exper Med*, *100* 321, 1954
47b ——— Studies on entry and egress of poliomyelitic infection VIII The relation of viremia to invasion of the central nervous system *J Exper Med*, *101* 383, 1955

48 ——— Poliocidal activity of some common antiseptics *Pediatrics* 12 637, 1953

49 Faber, H K, Silverberg, R J, and Dong, L Poliomyelitis in the cynomolgus monkey II Resistance to the spread of infection in the central nervous system following exposures of the mucous membranes to virus, with comments on non paralytic poliomyelitis *J Exper Med*, 78 519, 1943

50 ———: Poliomyelitis in the cynomolgus monkey III Infection by inhalation of droplet nuclei and the nasopharyngeal portal of entry, with a note on this mode of infection in rhesus *J Exper Med*, 80 39, 1944

51 ———. Poliomyelitis in the cynomolgus monkey IV Further observations on exposures confined to the stomach and intestines with notes on the fecal excretion of virus *J Exper Med*, 88 65 1948

52 Faber, H K, Dong L, and Silverberg R J A search for poliomyelitis virus in house dust and a study of the effects of drying on the stability of the virus *J Infect Dis*, 88 180, 1951

53 Faber, H K, McNaught R C Silverberg R J and Dong L Experimental production of post tonsillectomy bulbar poliomyelitis *Proc Soc Exper Biol & Med*, 77 532 1951

54 Faber, H K, and Rather L J Personal observations on histology of mesenteric lymph nodes after feeding virus Unpublished data 1953

55 Faber, H K, and Silverberg R J Experimental air borne infection with poliomyelitis virus *Science*, 94 566, 1941

56 ——— Pathway of invasion in a cynomolgus monkey after oral application of poliomyelitis virus *Science*, 96 473 1942

57 ——— A neuropathological study of acute human poliomyelitis with special reference to the initial lesion and to various potential portals of entry *J Exper Med*, 83 329, 1946

58 Faber, H K, and Silverberg R J Experiments on invasion of the CNS after tonsillectomy in cynomolgus monkeys Unpublished data, 1950 51

59 ——— Personal observations Partly unpublished see also references 50, 55 and 58

60 Faber, H K, Silverberg, R J and Dong L Excretion of poliomyelitis virus *Proc Soc Exper Biol & Med*, 66 103 1947

61 ——— Poliomyelitis in Philippine cynomolgus monkeys after simple feeding *Am J Hyg* 49 94 1949

62 ——— Studies on the entry and egress of poliomyelitis infection I Neurotropic infection in apparently healthy monkeys following casual exposure *J Exper Med*, 91 417 1950

63 ——— Studies on entry and egress of poliomyelitis infection II Entry and spread after exposure of the trigeminal nerve *J Exper Med* 91 519, 1950

64 Faber, H. K., Silverberg, R. J., Luz, L. A., and Dong, L.: Studies on entry and egress of poliomyelitis infection III Excretion of the virus during the presymptomatic period in parenterally inoculated monkeys *J. Exper. Med.*, *92* 571, 1950
65 Faber, H. K., Silberberg, R. J., and Dong, L.: Studies on entry and egress of poliomyelitic infection IV Atraumatic oral entry distribution of lesions and virus during the incubation period with notes on asymptomatic poliomyelitis *J. Exper. Med.*, *94* 455, 1951
66 ——— Studies on entry and egress of poliomyelitic infection V Entry after simple feeding with notes on viremia *J. Exper. Med.*, *97* 69, 1953
67 ——— Studies on entry and egress of poliomyelitic infection VI Centrifugal spread of the virus into peripheral nerve with notes on its possible implications *J. Exper. Med.* *97*.155, 1953
68. ——— Personal observations on CNS lesions in cynomolgus monkeys during preparalytic stage Unpublished data.
69 Fairbrother, R. W., and Hurst, E. W. The pathogenesis of, and propagation of the virus in, experimental poliomyelitis *J. Path. & Bact.* *33*.17, 1930
70 Flexner, S., and Amoss, H. L.: Localization of the virus and pathogenesis of epidemic poliomyelitis *J. Exper. Med.*, *20* 249, 1914
71 ——— The passage of neutralizing substances for the blood into the cerebrospinal fluid in poliomyelitis *J. Exper. Med.*, *25* 499, 1917
72 ——— The relation of the meninges and choroid plexus to poliomyelitic infection *J. Exper. Med.* *25* 525, 1917
73 Flexner, S., and Clark, P. F. Contamination of the fly with poliomyelitis virus *J.A.M.A.*, *56* 1717, 1911
74 ——— Epidemic poliomyelitis Eleventh note relation of the virus to the tonsils, blood and cerebrospinal fluid, races of the virus *J.A.M.A.*, *57* 1685, 1911.
75 Flexner, S., Peabody, F. W., and Draper, G. Epidemic poliomyelitis: visceral lesions of human cases *J.A.M.A.*, *58* 109, 1912
76 Follis, R. H., Jr., Orient Keiles, E., and McCollum, F. V. The production of cardiac and renal lesions in rats by a diet extremely deficient in potassium *Am. J. Path.*, *18* 29, 1942
77 Follis, R. H., Jr. The pathology of potassium deficiency *Journal-Lancet* *84* 241, 1953
78 Francis, T. Jr., Brown, G. C., and Penner, L. R. Search for extra human sources of poliomyelitis virus *J.A.M.A.*, *136* 1088, 1948
79 Frøvig, A. G. Poliomyelitis in mother and newborn infant in a family with multiple cases *Norsk mag. laegevidensk.*, *34* 1115, 1947.
80 Gebhardt, L. P. and Woodie, J. D. Possible dissemination of poliomyelitis virus by carriers *Am. J. Dis. Child.*, *76* 192, 1948
81 George, J., Hilden, T., and Vimtrap, B.: Cardiac involvement in poliomyelitis. *Ugesk. laeger*, *115* 886, 1953

82 Goldstein, D M, Hammon, W M, and Viets, H R: An outb d of poliencephalitis among navy cadets, possibly food b J.A.M.A, 131 569, 1946.

83 Gondol I I, and McBryde Heart failure in Addison's disease w myocardial changes of potassium deficiency J. Clin Endocrin 30, 1944

84 Goodpasture, F W Symposium on poliomyelitis the patholog poliomyelitis J.A.M.A, 117 273, 1941

85 —— Infantile Paralysis A Symposium, New York, The Nat Foundation for Infantile Paralysis, Inc., 1941, Lecture 4

86 ——: Virus infection of the mammalian fetus Science, 95 391 194

87 Gore, I, and Saphir, O Myocarditis A classification of 1402 c se Am Heart J, 34 827, 1947.

88 Guzzetti, H U Betrachtungen zur Poliomyelitis des Hirnstam Deutsche Ztschr Nervenh, 131 29, 1933

89 Hammon, W McD., Corell, L L, Wehrle, P F, and Stokes, J Jr Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis 4 Final report of results based on clinical diag nosis J.A.M.A, 151 1272, 1953

90 Hammon, W McD: Limitations in the use of gamma globulin in poliomyelitis Am J M Sc, 226 125, 1953

91 Harbitz, F, and Scheel, O Akute Poliomyelitis und verwandte Krankheiten Pathologisch Anatomische Untersuchungen aus den Epidemien in Norwegen, 1903-1906 Christiania, Dybwad 1907

92 ——: Epidemic acute poliomyelitis in Norway in the years 1903 to 1906 Results of anatomic investigation of nineteen cases of acute poliomyelitis and kindred conditions J.A.M.A, 49 1420, 1907

93 Harmon, P H, and Krigsten, W M Tests for the blood CNS barrier in experimental poliomyelitis Proc Soc Exper Biol & Med, 36 240, 1937

94 Havens, W P, and Paul J R: Infectious hepatitis and serum hepatitis in Viral and Rickettsial Infections of Man, Ed by T M Rivers, 2nd Ed Philadelphia, Lippincott, 1952, Chap 15

95 Horanyi Hechst, B, Zur histopathologie der menschlichen Poliomyelitis acuta anterior Deutsche Ztschr Nervenh, 137 1, 1935

96 Horsfall, F. L Influenza in Rivers Viral and Rickettsial Infections of Man, 2nd Ed Philadelphia, Lippincott, 1952, p 409

97 —— Prospects for the control of viral diseases by chemical agents Canad M A. J, 60 159, 1949

98 Horsfall, F L, Jr External factors influencing virus In Poliomyelitis Papers and Discussions, Second International Poliomyelitis Conference Philadelphia, Lippincott, 1952, p 20

99 Horstmann, D M Clinical aspects of acute poliomyelitis Am J Med, 6 555, 1949

100 ——: Poliomyelitis virus in blood of orally infected monkeys and chimpanzees *Proc Soc. Exper Biol & Med., 79*:417, 1952.
101 —— The epidemiology and pathogenesis of poliomyelitis *Bull New York Acad Med, 29* 910, 1953
102. ——. Viremia in poliomyelitis *Bull New York Acad Med, 29* 736, 1953
103 Horstmann, D M, and McCollum, R W· Poliomyelitis virus in human blood during the "minor illness" and the asymptomatic infection *Proc. Soc Exper Biol & Med, 82* 431, 1953
104 Horstmann, D M, Ward, R, and Melnick, J. L The isolation of poliomyelitis virus from human extraneural sources III Persistence of virus in stools after acute infection *J Clin Investigation, 25*:278, 1946
105 Howe, H A Epidemiology of poliomyelitis in the light of modern research *Am J Med, 6* 535, 1949
106 Howe, H A, and Bodian, D Portals of entry of poliomyelitis virus in the chimpanzee, *Proc Soc Exper Biol & Med, 43* 718, 1940
107. —— Poliomyelitis in the chimpanzee a clinical-pathological study *Bull Johns Hopkins Hosp, 69* 149, 1941.
108 —— Neural Mechanisms in Poliomyelitis New York, Commonwealth Fund, 1942
109 —— Poliomyelitis in the cynomolgus monkey following oral inoculation *Am J Hyg, 48* 99, 1948
110 Howe, H A, Bodian, D, and Morgan, I. M Subclinical poliomyelitis in the chimpanzee and its relation to alimentary reinfection *Am. J Hyg, 51* 85, 1950
111. Howe, H A, and Peele, T The pia arachnoid as a barrier in experimental poliomyelitis *Proc Soc Exper Biol & Med, 41* 545, 1939
112 Hurst E W The occurrence of intranuclear inclusions in the nerve cells in poliomyelitis *J Path & Bact, 34* 331, 1931
113 —— Further observations on the pathogenesis of experimental poliomyelitis intrathecal inoculation of the virus *J Path. & Bact. 35* 41, 1932
114 International Committee for the Study of Infantile Paralysis Poliomyelitis Baltimore, Williams & Wilkins, 1932, pp 74 75
115 Jennison, M W Atomizing of mouth and nose secretions into the air as revealed by high speed photography in Aerobiology,' Ed by F R Moulton Washington, DC, Am Assn for the Advancement of Science, 1942, pp 106 128
116 Johns, A W Cases of acute poliomyelitis *Med Officer, 80* 230, 1948
117 Johnson, J F, and Stimson, P M Clinical poliomyelitis in the early neonatal period *J Pediat, 40* 733, 1952
118 Jordi, A Peripheral neuritis in experimental poliomyelitis notes on the nature of the first pathologic changes in the spinal cord *J Infect Dis, 49* 550, 1931

119 Jungeblut, C W Newer knowledge on the pathogenesis of poliomyelitis *J Pediat*, *37* 109, 1950

120 Jungeblut, C. W., and Stevens, M A: Attempts to isolate poliomyelitis virus from the paralyzed muscle of patients during the acute stage of the disease *Am J Clin Path*., *20* 701, 1950

121 Jungeblut, C W, and Thompson, R Does the virus of poliomyelitis survive in the monkey testicle? *Proc Soc Exper Biol. & Med*, *27* 819, 1930.

122 Kausche, G.-A, and Hoffmann-Burling, H: Zur Morphologie des Achsenzylinders beim Warmblüter und seine Beziehungen zu neurotropen Viren *Arch. ges. Virusforschung*, *4* 421, 1951

123 Kempf, F F, and Soule, M H Attempts to demonstrate poliomyelitis in extraneural tissues *Proc Soc Exper Biol & Med*, *43* 476, 1940

124 Kessel, J F., and Moore, F J Occurrence of poliomyelitis virus in tonsils and stools of non-contacts during an interepidemic period *Am J Hyg*, *41* 25, 1945

125 Kessel, J F, Moore, F J, and Stimpert, F D: The occurrence of poliomyelitis virus in autopsies, patients and contacts *J Exper Med*, *74* 601, 1941

126 Kitaoka, M, and Mitamura, T Experimental transmission of poliomyelitis virus by mosquitoes in rhesus monkeys *Jap M J*, *3* 167, 1950

127 Kling, C, Levaditi C and Lepine, P La penetration du virus poliomyélitique à travers la muqueuse du tube digestif chez le singe et sa conservation dans l'eau *Bull Acad Med*, *102* 158, 1929

128 Koprowski, H., Norton, T W, and McDermott, W Isolation of poliomyelitis virus from human serum by direct inoculation into the laboratory mouse *Pub Health Rep*, *62* 1467, 1947

129 Koprowski, H, Jervis, G A, and Norton, T W Immune responses in human volunteers upon oral administration of a rodent adapted strain of poliomyelitis virus *Am J Hyg*, *55* 108, 1952

130 Koprowski, H, Jervis, G A, Norton, T W, and Nelson D J Further studies on oral administration of living poliomyelitis virus to human subjects *Proc Soc Exper Biol & Med*, *82* 277, 1953

131 Koprowski, H Discussion of classification and nomenclature of the poliomyelitis virus group, in Virus and Rickettsial Classification and Nomenclature *Ann New York Acad Med*, *56* 601, 1953

132 Kreibich, H, and Wolf, W Ueber einen Fall von diaplazentar erfolgter Poliomyelitisinfektion des Feten im 9 Schangerschafts monat *Zentralbl Gynak*, *72* 694, 1950

133 Krieg, W J *Functional Neuroanatomy* Philadelphia, Blakiston, 1942

134 Landon, J F, and Smith, L W *Poliomyelitis* New York, Macmillan, 1934

135 Landsteiner, K, and Levaditi, C La transmission de la paralysie infantile aux singes, *Compt. rend Soc biol*, *67*:592, 1909

136 Leake, J P Poliomyelitis following vaccination against this disease *J A M A*, *105* 2152, 1935

137. Leiner, C, and von Wiesner, R Experimentelle Untersuchungen über Poliomyelitis acuta anterior. *Wien klin Wchnschr*, *22* 1698, 1909

138 *Lennette, E H Studies on the role of the spleen in experimental* poliomyelitis *J Exper Med*, *66* 519, 1937.

139 Levaditi, C, and Danulesco, V Mode de contagion de la poliomyélite *Compt rend. Soc biol*, *72* 513, 1912

140 Levaditi, C, Kling, C, and Hornus, G Transmission expérimentale de la poliomyélite par la voie digestive *Compt rend Soc. biol*, *112* 43, 1933

141 Lipari, M: A milkborne poliomyelitis episode *New York State J Med*, *51* 362, 1951

142 Lippard, V W, Schloss, O M, and Johnson, P A Immune reactions induced in infants by intestinal absorption of incompletely digested cow's milk protein *Am J Dis Child*, *51* 562, 1936

143 Marinesco, G, Manicatide, M, and State Draganesco Étude clinico therapeutique et anatomo pathologique sur l'epidémie de paralysie infantile qui a sevi en Roumanie pendant l'année 1927. *Ann Inst Pasteur*, *43* 223, 1929

144 Martin, J K Local paralysis in children after injections *Arch Dis Childhood*, *25* 1, 1950

145 Maximow, A A, and Bloom, W. *A Textbook of Histology*, 5th Ed Philadelphia, Saunders, 1948

146 McCloskey, B P (a) The relation of prophylactic inoculations to the onset of poliomyelitis *Lancet*, *1* 659, 1950 (b) The relation of prophylactic inoculations to the onset of poliomyelitis a study of 620 cases in the Victorian epidemic of poliomyelitis in 1949 *M J Australia*, *1* 612, 1951

147 Melnick J L Poliomyelitis in the blood stream in the experimental disease *Proc Soc Exper Biol & Med*, *58* 14, 1945

148 ——— The recovery of poliomyelitis virus from the stools of experimentally infected monkeys and chimpanzees *J Immunol*, *53* 277, 1946

149 ——— Differences in the degree of infectiousness of two related strains of poliomyelitis virus following their oral administration to monkeys *J Immunol* *67* 219, 1951

150 Melnick, J L, and Horstmann, D. M.[illegible] immu[illegible] mye-litis in chimpanzees following [illegible] ection [illegible] ed, *85* 287 1947

151 Melnick, J L, and Ledinko, N [illegible] bodies against the three types [illegible]

epidemic period The ratio of inapparent infection to clinical poliomyelitis *Am J Hyg*, 58 207, 1953

152 Melnick, J L, Rhian, M, Warren, J, and Breese, S S, Jr The size of Coxsackie viruses and Lansing poliomyelitis virus determined by sedimentation and ultrafiltration *J Immunol*, 67 151, 1951

153 Moskowitz, L Follow up study of seventy five cases of nonparalytic poliomyelitis *J A M A*, 152 1505, 1953

154 Nicolau, S, and Galloway, I Borna disease and enzootic encephalomyelitis of sheep and cattle Spec Rep Series No 121, Med Res Council, London, 1928

155 Nicolau, S, Dimanesco-Nicolau, O, and Galloway, I A Étude sur les septinévrites à ultravirus neurotropes *Ann Inst Pasteur*, 43 1, 1929

156 Neustaedter, M and Thro W C Experimental poliomyelitis produced in monkeys from the dust of the sick room, a further contribution *New York State J Med*, 94 613, 1911

157 O Leary, J L Heinbecker, P, and Bishop, G H Nerve degeneration in poliomyelitis IV Physiologic and histologic studies on the roots and nerves supplying paralyzed extremities of monkeys during acute poliomyelitis *Arch Neurol & Psychiat*, 28 272, 1932

158 Paul J R Current knowledge and trends in poliomyelitis *Merck Report*, 61 3, 1952

159 Paul, J R, Trask, J D, Melnick, J L and Casey A E The detection of poliomyelitis virus in flies *Science*, 94 395, 1941

160 Peabody F W, Draper, G, and Dochez A R A clinical study of acute poliomyelitis Monographs of the Rockefeller Institute No 4, New York, The Rockefeller Institute for Medical Research, 1912

161 Ranson, S W The Anatomy of the Nervous System 7th Ed Philadelphia, Saunders, 1943

162 Reagan, R L, Schenck D M and Brueckner, A L Morphological observations by electron microscopy of the Brunhilde strain of poliomyelitis virus *J Infect Dis*, 86 295 1950

163 Reagan, R L, Schenck D M, and Brueckner A L Morphological observations of the Leon strain of poliomyelitis virus *Proc Soc Exper Biol & Med*, 77 42 1951

164 Reagan, R L and Brueckner A L Morphological observations by electron microscopy of the Lansing strain of poliomyelitis virus after propagation in the Swiss albino mouse *Texas Rep Biol & Med*, 10 425 1952

165 Rhodes, A J Classification and nomenclature of the poliomyelitis viruses, In *Virus and Rickettsial Classification and Nomenclature Ann New York Acad Sc*, 56 396 1953

166 Riley, H A An Atlas of the Basal Ganglia Brain Stem and Spinal Cord Baltimore, Williams and Wilkins, 1943

167 Rissler, J Zur Kenntniss der Veränderungen des Nervensystems bei Poliomyelitis anterior acuta *Nord med. Arkiv*, *20* I, 1888.

168 Sabin, A B · Experimental poliomyelitis by the tonsillopharyngeal route with special reference to the influence of tonsillectomy on the development of bulbar poliomyelitis *J. A M A*, *111* 605, 1938

169 ——— The olfactory bulbs in human poliomyelitis *Am J Dis Child*, *60* 1313, 1940

170 ———. Transmission of poliomyelitis virus analysis of differing interpretations and concepts, practical implications *Chicago M Soc Bull*, *54* 163, 1951.

171 ——— Present status and future possibilities of a vaccine for the control of poliomyelitis *Am. J Dis Child*, *86* 301, 1953

172 Sabin, A B, Hennessen, W A, and Warren, J · Ultrafiltration and electron microscopy of three types of poliomyelitis virus propagated in tissue culture *Proc Soc Exper. Biol. & Med*, *85* 359, 1954

173 Sabin, A B, and Ward, R Nature of non paralytic and transitory paralytic poliomyelitis in rhesus monkeys inoculated with human virus *J Exper Med*, *73* 757, 1941.

174 ——— The natural history of human poliomyelitis I Distribution of virus in nervous and non nervous tissues. *J Exper Med*, *73* 771, 1941

175 ——— The natural history of human poliomyelitis II Elimination of the virus *J Exper Med*, *74* 519, 1941

176 ——— Flies as carriers of poliomyelitis virus in urban epidemics *Science*, *94* 590, 1941

177 ——— The behavior of poliomyelitis virus in cynomolgus monkeys infected by the oral route *J Bact*, *43* 86, 1942

178 Saddington, R S An intravascular lesion in poliomyelitis induced by feeding in Macacus cynomolgus *Proc Exper Biol & Med*, *29* 838, 1932

179 Salk, J E Studies in human subjects on active immunization against poliomyelitis I A preliminary report of experiments in progress *J A M A*, *151* 1081, 1953

180 ——— Recent studies on immunization against poliomyelitis *Pediatrics*, *12* 471, 1953

180a ——— Studies in human subjects on active immunization against poliomyelitis II A practical means for inducing and maintaining antibody formation *Am J Pub Health*, *44* 991, 1954

181 Saphir O Visceral lesions in poliomyelitis *Am J Path*, *21* 99, 1945

182 Schultz, E W, and Gebhardt, L P Prevention of intranasally inoculated poliomyelitis in monkeys by previous intranasal irrigation with chemical agents *Proc Soc Exper Biol & Med*, *34* 133, 1936

183 Schwartzman, G (Editor) *The Effect of Cortisone upon Infection and Resistance*, Chapter 14 Schwartzman, G, and Aronson, S M Alteration of experimental poliomyelitis by means of cortisone with reference to other viruses, New York, Columbia University Press, 1953

184 Selye, H The general adaptation syndrome and the diseases of adaptation *J Clin Endocrinol*, *4* 208, 1944

185 Shaw, E B, and Levin, M The infrequent incidence of non paralytic poliomyelitis *J Pediat*, *44* 237, 1954

186 Smith, W M, Chambers, V C, and Evans, C A Growth of neurotropic viruses in extraneural tissues IV Poliomyelitis virus in human testicular tissue in vitro *Proc Soc Exper Biol & Med*, *76* 696, 1951

187 Sommers, S C, Wilson, J C, and Hartman F W Lymphoid lesions in poliomyelitis *J Exper Med*, *93* 505, 1951

188 Spielmeyer, W Histopathologie und Pathogenese der Poliomyelitis *Ztschr ges Neurol u Psychiatr*, *142* 159, 1932

189 Swan, C The anatomical distribution and character of the lesions of poliomyelitis with special reference to the type of cell affected and to the portal of entry of the virus *Australian J Exper Biol & Med Sc*, *17* 345, 1939

189a Swarts, C L, and Kercher, E F A fatal case of poliomyelitis in a newborn infant delivered by cesarean section following maternal death due to poliomyelitis *Pediatrics*, *14* 235, 1954

190 Taylor, C F *Infantile Paralysis, and its Attendant Deformities* Philadelphia, Lippincott, 1867

191 Topley and Wilson's Principles of Bacteriology and Immunity, Vol 2 3rd Ed Baltimore, Williams and Wilkins, 1946 pp 1102 1105

192 Trask, J D, and Paul, J R The skin infectivity of poliomyelitis virus *Science*, *87* 44 1938

193 Turner, T B, Hollander D H, Buckley S, Kokko, U P and Winsor, C P Age incidence and seasonal development of neutralizing antibodies to Lansing poliomyelitis virus *Am J Hyg*, *52* 323, 1950

194 von Magnus, H and Melnick J L Antibody responses in monkeys following oral administration of poliomyelitis virus *J Immunol*, *60* 583, 1948

195 Walzer, M A direct method of demonstrating the absorption of incompletely digested proteins in normal human beings *J Immunol*, *11* 249, 1926

196 Ward R Horstmann, D M and Melnick J L The isolation of poliomyelitis from extraneural sources IV Search for virus in the blood of patients *J Clin Investigation*, *25* 284 1946

197 Ward, R, Melnick, J L, and Horstmann, D M Poliomyelitis virus

in fly-contaminated food collected at an epidemic *Science, 101:*191, 1945.

198 Ward, R, and Walters, B · Elimination of poliomyelitis virus from the human mouth and nose *Bull Johns Hopkins Hosp*, *80* 98, 1947.

199 Weaver, H M, and Steiner, G Acute anterior poliomyelitis during pregnancy *Am J Obst and Gynec*, *47* 495, 1944

200 Webster, L T *Rabies* New York, Macmillan, 1942

201 Weed, L H · Studies on cerebrospinal fluid IV. The dual source of cerebrospinal fluid *J. Med. Res*, 31 (n ss 26) 93, 1914

202 Weed, L H, and McKibben, P S Experimental alteration of brain bulk *Am J. Physiol*, *48* 531, 1919

203 Weinstein, L and Shelokov, A · Cardiovascular manifestations in acute poliomyelitis *New England J Med*, *244* 281, 1951

204 Weiss, P, and Hiscoe, H B Experiments on the mechanism of nerve growth *J Exper Zool*, *107* 315, 1948

205 Weller, T H, Enders, J F, and Robbins, F. C · Studies on the cultivation of poliomyelitis viruses in tissue culture I The propagation of poliomyelitis viruses in suspended cell cultures of various human tissues *J Immunol*, *69* 645, 1952

206 Wells, W F On air-borne infection II Droplets and droplet nuclei *Am J Hyg*, *20* 611, 1934

207 Wells, W F, and Henle, W Experimental air borne disease Quantitative inoculation by inhalation of influenza virus. *Proc. Soc Exper Biol & Med*, *48* 298, 1941

208 Wells, W F, and Lurie, M. B. Experimental air borne disease quantitative natural respiratory contagion of tuberculosis *Am J Hyg*, *34* 21, 1941

209 Wells, W F, Wells, M W, and Mudd, S · Infection of air *Am J Pub Health*, *29* 863, 1939

210 Wells, W F, Wells, M W, and Wilder, T S The environmental control of epidemic contagion I An epidemiologic study of radiant disinfection in day schools *Am J Hyg*, *35* 97, 1942

211 Wenner, H A, and Rabe, E F The recovery of virus from regional lymph nodes of fatal human cases of poliomyelitis *Am J M Sc*, *222* 292, 1951

212 Wislocki, G B, and Putnam, T J Further observations on the anatomy and physiology of the areae postremae *Anat Rec*, *27*·151, 1924

213 Wood W, Clark, F M, McKendry, J B J, and Rhodes, A J Studies on passive immunity in poliomyelitis Lansing antibody levels in humans after gamma globulin administration *Proc Soc Exper Biol & Med*, *80* 522, 1952

214 Yoffey J M, and Drinker C K Poliomyelitis and lymphatic apparatus *J Exper Med*, *70* 83, 1939

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This Book

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By HAROLD K. FABER, M.D

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